

# ISOPYRAZAM

First draft prepared by  
Jürg Zarn<sup>1</sup> and Alan Boobis<sup>2</sup>

<sup>1</sup> Nutritional and Toxicological Risks Section, Swiss Federal Office of Public Health,  
Zurich, Switzerland

<sup>2</sup> Faculty of Medicine, Imperial College London, London, England

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## Explanation

Isopyrazam is the provisional International Organization for Standardization (ISO)–approved name for a mixture of two *syn* isomers of 3-(difluoromethyl)-1-methyl-*N*-[(1*RS*,4*SR*,9*RS*)-1,2,3,4-tetrahydro-9-isopropyl-1,4-methanonaphthalen-5-yl]pyrazole-4-carboxamide and two *anti* isomers

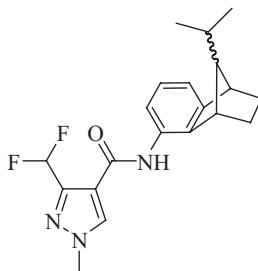
of 3-(difluoromethyl)-1-methyl-*N*-[(1*RS*,4*SR*,9*SR*)-1,2,3,4-tetrahydro-9-isopropyl-1,4-methanonaphthalen-5-yl]pyrazole-4-carboxamide (Chemical Abstracts Service No. 881685-58-1). Technical isopyrazam contains *syn* (SYN534969) and *anti* (SYN534968) isomers at ratios from 70:30 to 100:0 *syn:anti*.

Isopyrazam is a new broad-spectrum fungicide of the *ortho*-substituted phenyl amides, acting by inhibition of succinate dehydrogenase. It has not been evaluated previously by the Joint FAO/WHO Meeting on Pesticide Residues and was reviewed at the present Meeting at the request of the Forty-second Session of the Codex Committee on Pesticide Residues.

All the pivotal studies met the requirements of the relevant Organisation for Economic Co-operation and Development or national test guidelines and were certified as complying with good laboratory practice.

The chemical structure of isopyrazam is shown in Figure 1.

**Figure 1. Chemical structure of isopyrazam**



## Evaluation for acceptable daily intake

### 1. Biochemical aspects

#### 1.1 Absorption, distribution and excretion

A whole-body autoradiography and expired air study in male and female Han Wistar rats was performed. Single doses of 97.8% radiochemically pure [pyrazole-5-<sup>14</sup>C]isopyrazam (labelled 92.25:7.75 *syn:anti*; non-labelled 92.8:7.2 *syn:anti*) at 2.5 mg/kg body weight (bw) (3.73 MBq/mg) or 250 mg/kg bw (0.0396 MBq/mg) were administered by oral gavage to one animal of each sex in the expired air study group and to four animals of each sex in the whole-body autoradiography study group. The radioactivity in expired air, urine, faeces and cage wash was determined at 10, 24, 34 (air only) and 48 hours after dosing. The radioactivity remaining in the blood, plasma, gastrointestinal tract plus contents and residual carcass of each rat was also determined. In the whole-body autoradiography group, subgroups of one male and one female rat were terminated at 2, 24 and 48 hours after dosing, and the distribution of radioactivity in the carcass was determined by whole-body autoradiography.

The recovery of radioactivity was 96–98% in the 2.5 mg/kg bw groups and 76–87% in the 250 mg/kg bw groups. After 48 hours, the total radioactive residues (TRR) in expired air were less than 0.05% of the dose in both male and female rats at both dose levels. At both dose levels, 2 hours after dosing, the majority of the radioactivity was found in the stomach, liver, gastrointestinal tract and kidney. Lower concentrations were found in the Harderian gland, abdominal fat and brown fat. Low levels of radioactivity were seen in heart, lungs, spleen, pancreas, adrenals, thymus, salivary glands, testes, ovaries, uterus, brain, spinal cord and muscle. Radioactivity was not seen in the thyroid, eye

or bone. By 24 hours after dosing, very little radioactivity remained in the bodies of the rats. TRR depletion was slower in high-dose females than in low-dose females. The majority of the remaining radioactivity was found in the gastrointestinal tract and stomach, with lower levels in the liver and kidney (Silcock, 2007).

To investigate the pharmacokinetics of isopyrazam, single doses of 98.6% radiochemically pure [pyrazole-5-<sup>14</sup>C]isopyrazam (labelled 98.8:1.2 *syn:anti*; non-labelled 92.8:7.2 *syn:anti*) at 1 mg/kg bw (5.03 MBq/mg) or 75 mg/kg bw (0.07 MBq/mg) were administered by oral gavage to 13 Han Wistar rats of each sex per dose. For pharmacokinetic analyses in blood and plasma, nine animals of each sex per dose group were allocated to three blood sampling groups covering 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 12, 24 and 48 hours after dosing. To obtain plasma for metabolic profiling, two animals of each sex per dose were terminated at 3 and 6 hours after dosing, and plasma was analysed in a separate study (Green, 2008).

Maximum blood and plasma TRR concentrations were achieved at 3–6 hours in male and female rats after dosing of 1 or 75 mg/kg bw (Table 1). The mean terminal half-lives in blood and plasma of low-dose females were similar (4.6 and 4.8 hours). In males, terminal half-life and area under the curve ( $AUC_{0-\infty}$ ) values could not be calculated because no terminal mono-exponential phase could be identified. For the same reason, the half-life and  $AUC_{0-\infty}$  in plasma of high-dose females could not be calculated. However, excretion was virtually complete within 48 hours (Figures 2 and 3). In plasma and blood at both dose levels, females had higher TRR concentrations than males. Radioactivity was excreted via the faeces, accounting for 70.9% and 78.0% at the two dose levels, respectively. Excretion via the urine was 12.9% and 18.0% of the administered dose at the two dose levels, respectively (Allan, 2008a).

To investigate the excretion pattern and tissue distribution of isopyrazam, single doses of 99.4% radiochemically pure [pyrazole-5-<sup>14</sup>C]isopyrazam (labelled 98.8:1.2 *syn:anti*; non-labelled 92.8:7.2 *syn:anti*) at 1 mg/kg bw (4.88 MBq/mg) and 75 mg/kg bw (0.06 MBq/mg) were administered by oral gavage to four Han Wistar rats of each sex per dose. The age of the high-dose animals was 6–7 weeks, and that of the low-dose animals was 13 weeks. The excreta of the high-dose animals were also used in another study (Allan, 2008a). Urine, faeces and cage washes were collected over 7 days, and animals were then killed. TRR in the organs were analysed.

Recovery of TRR was 106.0–106.4% in the low-dose group and 95.7–100.5% in the high-dose group. In the low-dose group, excretion was virtually complete within 48 hours; 77.3–83.0% of TRR was recovered in faeces, and 19.6–27.1% in urine. In the high-dose group, 89.8–92.2% of the administered dose was excreted within 48 hours. At 7 days post-dosing, 78.5–79.4% was excreted via faeces and 13.3–17.5% via urine. At day 7 post-dosing, animals of both sexes in the low-dose group had 0.005–0.010 µg equivalents (eq) per gram in the liver and 0.002–0.003 µg eq/g in the kidney. The concentration of TRR in all other organs was below the limit of detection. In the high-dose group, animals of both sexes had 0.284–0.586 µg eq/g in the liver, and a concentration of 0.121 µg eq/g was measured in the kidneys of males. The concentration of TRR in all other organs was below the limit of detection (tissue limit of detection not stated) (Shaw, 2008b).

To investigate the tissue depletion of isopyrazam from tissues, single doses of 99.4% radiochemically pure [pyrazole-5-<sup>14</sup>C]isopyrazam (labelled 98.8:1.2 *syn:anti*; non-labelled 92.8:7.2 *syn:anti*) at 1 mg/kg bw (4.88 MBq/mg) or 75 mg/kg bw (0.06 MBq/mg) were administered by oral gavage to 15 Han Wistar rats of each sex per dose. At 6, 12, 24, 48 and 96 hours post-dosing in the low-dose group and 10, 18, 24, 48 and 96 hours post-dosing in the high-dose group, three animals of each sex per dose were killed, and organs, tissues and body fluids were retained for TRR analysis.

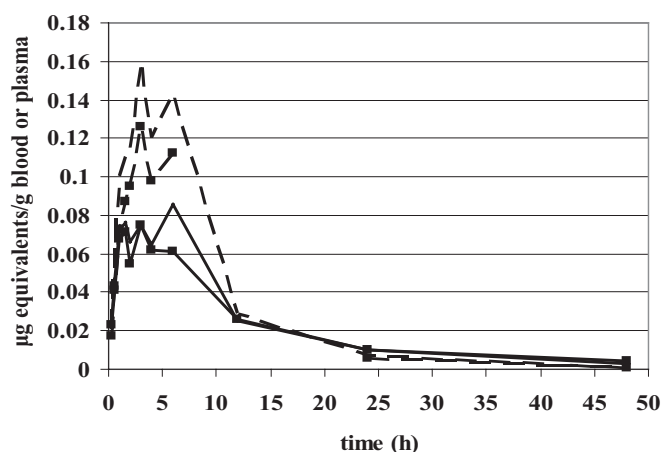
**Table 1. Plasma and blood pharmacokinetic parameters in rats following single oral administration of isopyrazam at 1 or 75 mg/kg bw**

|                                                              | 1 mg/kg bw |         |        |         | 75 mg/kg bw |         |        |         |
|--------------------------------------------------------------|------------|---------|--------|---------|-------------|---------|--------|---------|
|                                                              | Blood      |         | Plasma |         | Blood       |         | Plasma |         |
|                                                              | Males      | Females | Males  | Females | Males       | Females | Males  | Females |
| $C_{\max}$ ( $\mu\text{g eq/ml}$ )                           | 0.075      | 0.126   | 0.0857 | 0.160   | 6.31        | 12.2    | 7.56   | 17.7    |
| $T_{\max}$ (h)                                               | 3          | 3       | 6      | 3       | 3           | 4       | 3      | 4       |
| $\text{AUC}_{0-t}$ ( $\mu\text{g eq}\cdot\text{h/ml}$ )      | 1.00       | 1.62    | 1.14   | 1.44    | 96.9        | 210     | 81.4   | 207     |
| $\text{AUC}_{0-\infty}$ ( $\mu\text{g eq}\cdot\text{h/ml}$ ) | NC         | 1.67    | NC     | 1.49    | 98.7        | 211     | 82.7   | NC      |
| $t_{1/2}$ (h)                                                | NC         | 4.81    | NC     | 4.60    | 8.68        | 6.21    | 7.52   | NC      |

From Allan (2008a)

AUC, area under the concentration–time curve;  $C_{\max}$ , peak concentration; eq, equivalents; NC, not calculated;  $t_{1/2}$ , half-life;  $T_{\max}$ , time to reach  $C_{\max}$

**Figure 2. Mean total radioactive residue concentrations in plasma and blood of male and female rats following a single oral administration of [pyrazole-5- $^{14}\text{C}$ ]isopyrazam at 1 mg/kg bw**

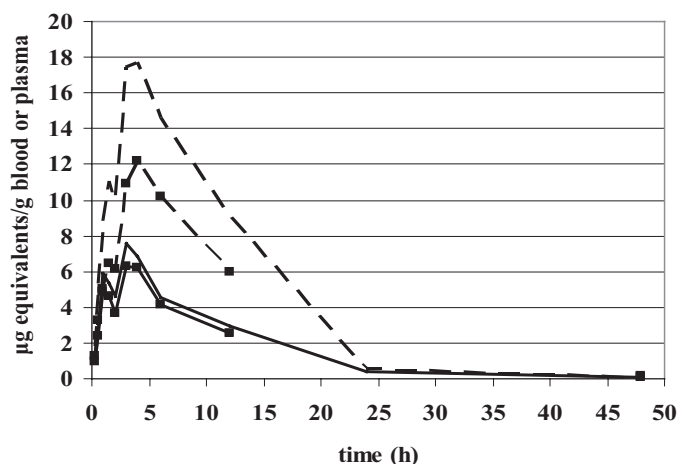


Solid line (males), dashed line (females), squares (blood), no symbols (plasma)

Source: Allan (2008a)

At 1 mg/kg bw, the highest levels of TRR were observed in both sexes at the first sampling time at 6 hours. In males at this time point, 3.1% of the dose was found in the carcass and 2.3% in liver; in females, the respective values were 7.1% and 2.6%. In males, the mean concentrations were 0.551  $\mu\text{g eq/g}$  in the liver, 0.310  $\mu\text{g eq/g}$  in the kidney and 0.196  $\mu\text{g eq/g}$  in the adrenals. In females, the respective values were 0.677  $\mu\text{g eq/g}$ , 0.397  $\mu\text{g eq/g}$  and 0.356  $\mu\text{g eq/g}$ . In both sexes, the concentration of TRR in tissues declined, retaining the relative organ distribution, and the initially higher TRR contents in female organs converged to the concentrations in males. At 96 hours post-dosing, the TRR remaining in the carcass accounted for approximately 0.5% of the dose. In male rats, the elimination half-lives for blood, heart, kidneys, liver and lungs were similar and were in the range of 29–53 hours. The elimination half-lives for plasma and bone mineral were 11 and 87 hours, respectively. In female rats, the elimination half-lives for bone mineral, brain and plasma were very similar and were in the range of 3–5 hours. The elimination half-lives for thymus, uterus, lungs, liver, renal fat, pancreas and kidneys were 10, 14, 23, 24, 53, 67 and 71 hours, respectively.

**Figure 3. Mean total radioactive residue concentrations in plasma and blood of male and female rats following a single oral administration of [pyrazole-5-<sup>14</sup>C]isoprazam at 75 mg/kg bw**



Solid line (males), dashed line (females), squares (blood), no symbols (plasma)  
Source: Allan (2008a)

At 75 mg/kg bw, the highest levels of TRR were observed in both sexes at the first sampling time at 10 hours. In males at this time point, 6.9% of the dose was found in the carcass and 2.5% in liver; in females, the respective values were 8.2% and 1.8%. In males, the mean concentrations were 53.5 µg eq/g in the liver, 17.0 µg eq/g in the kidney, 16.1 µg eq/g in the thyroid, 14.1 µg eq/g in the bone mineral and 13.8 µg eq/g in the adrenals. In females, 58.0 µg eq/g were found in the renal fat, 35.5 µg eq/g in the liver, 23.7 µg eq/g in the ovaries, 28.2 µg eq/g in the adrenals and 20.2 µg eq/g in the uterus. TRR levels in pancreas, kidneys, lungs, thyroid, heart and spleen were also higher than those observed in whole blood (6.13 µg eq/g). In both sexes, TRR levels in tissues declined, retaining the relative organ distribution. At 96 hours, detectable TRR levels in males and females were found in liver and renal fat and in males also in kidney, pancreas and lungs. In males, the remaining radioactivity accounted for 0.48% of the administered dose, and in females, it was barely quantifiable. In male rats, the elimination half-lives for adrenals, heart, muscle, plasma, spleen, testes and thymus were very similar and were in the range of 4–6 hours. The elimination half-lives for blood, lungs, renal fat and kidneys were 8, 24, 48 and 49 hours, respectively. In female rats, the elimination half-lives for adrenals, lungs, ovaries, plasma, spleen and thymus were very similar and were in the range of 3–5 hours. The elimination half-lives for muscle, pancreas, uterus and blood were also very similar and were in the range of 6–7 hours. The elimination half-lives for renal fat and liver were 17 and 29 hours, respectively (Allan, 2008b).

To investigate the excretion and tissue distribution of isoprazam, 30 male Han Wistar rats were administered by gavage daily doses of 1 mg/kg bw per day (0.954 MBq/mg) of 99.4% radiochemically pure [pyrazole-5-<sup>14</sup>C]isoprazam (labelled 98.8:1.2 *syn:anti*; non-labelled 92.8:7.2 *syn:anti*) for maximally 14 days. Twenty-four hours after 3, 7, 10 and 14 doses and 3, 7, 10, 14, 28 and 43 days after 14 doses, three animals per time point were killed. To analyse for TRR, organs and blood samples were collected. Additionally, for 24 hours after the first dose or for 24 hours after 14 doses, faeces and urine were collected.

The highest TRR levels were found in liver (0.361 µg eq/g), kidneys (0.121 µg eq/g) and blood (0.033 µg eq/g) at 24 hours after 14, 10 and 14 days, respectively. In most other organs, concentrations were below 0.05 µg eq/g. With the exception of the liver and renal fat, the steady-state TRR concentration in tissues was reached by 24 hours after dosing day 10. After cessation of dosing, the TRR concentration in organs decreased rapidly, and 14 days after dosing day 14, radioactivity was measurable only in liver and kidneys. In a 24-hour period after 1 day of exposure, 47.9% of the administered dose was excreted in faeces and 19.7% in urine. After a 14-day exposure period, 88.6% was excreted in faeces and 21.6% in urine within a collection period of 24 hours (Allan, 2008c).

To investigate the possibly different excretion profiles of *syn* and *anti* isopyrazam, single doses of 2 or 75 mg/kg bw of *syn* or *anti* [pyrazole-5-<sup>14</sup>C]isopyrazam were administered by oral gavage to four male and four female bile duct-cannulated Han Wistar rats. Unlabelled *syn* isomer (SYN534969) was 98.7% chemically pure, with a 99.3:0.7 *syn:anti* isomer ratio; the corresponding radiolabelled *syn* isomer was 98.4% radiochemically pure. Unlabelled *anti* isomer (SYN534968) was 98.4% chemically pure, with a 1:99 *syn:anti* isomer ratio; the corresponding radiolabelled *anti* isomer was 98.5% radiochemically pure (mean of two batches used). The low-dose *syn* isomer had a specific activity of 2.51 MBq/mg, and the low-dose *anti* isomer, 1.69 MBq/mg. The high doses of *syn* and *anti* isomers both had a specific activity of 0.068 MBq/mg. The female high-dose *syn* isomer group consisted of seven animals, and the male high-dose *anti* isomer group, five animals. Urine, bile, faeces and cage washes were collected at intervals over 3 days, after which time the rats were terminated. A terminal blood sample was removed, together with the gastrointestinal tract and residual carcass, and each sample taken was analysed for residual radioactivity.

Three high-dose *syn* isomer females were killed prior to study termination because of severe clinical signs observed in this group. Effects were attributed to the surgical procedure exacerbated by treatment, rather than to treatment with isopyrazam alone.

For the *anti* isomer, at 2 mg/kg bw, the major routes of elimination in both males and females were the bile (means of 38.3% and 56.1% of the administered dose in males and females, respectively) and the faeces (means of 32.5% and 28.3% of the administered dose in males and females, respectively), recovered over 3 days post-dosing (Table 2). Urinary excretion accounted for means of 22.1% and 12.2% of the dose in males and females, respectively. Absorption accounted for 60.6% of the dose in males and 68.6% of the dose in females, as calculated from the biliary and urinary excretion and TRR remaining in the carcass. At 75 mg/kg bw, the major routes of elimination in both males and females were the bile (means of 36.5% and 61.1% of the administered dose in males and females, respectively) and the faeces (means of 38.3% and 20.4% of the administered dose in males and females, respectively), recovered over 3 days post-dosing. Urinary excretion accounted for means of 16.3% and 15.9% of the dose in males and females, respectively. Absorption accounted for 53.0% of the dose in males and 77.2% of the dose in females, as calculated from the biliary and urinary excretion and TRR remaining in the carcass.

For the *syn* isomer, at 2 mg/kg bw, the major routes of elimination in both males and females were the bile (means of 56.3% and 48.9% of the administered dose in males and females, respectively) and the faeces (means of 23.8% and 19.8% of the administered dose in males and females, respectively), recovered over 3 days post-dosing. Urinary excretion accounted for means of 15.4% and 26.0% of the dose in males and females, respectively. Absorption accounted for 71.8% of the dose in males and 75.1% of the dose in females, as calculated from the biliary and urinary excretion and TRR remaining in the carcass. At 75 mg/kg bw, the major routes of elimination in both males and females were the bile (means of 58.0% and 41.6% of the administered dose in males and females, respectively) and the faeces (means of 34.1% and 48.3% of the administered dose in males and females, respectively), recovered over 3 days post-dosing. Urinary excretion accounted for means of 7.6% and 7.0% of the dose in males and females, respectively. Absorption accounted for 65.7% of the

**Table 2. Excretion of *syn* and *anti* isomers of isopyrazam in male and female rats at single doses of 2 and 75 mg/kg bw**

| Excreta                   | Excretion (% of administered dose) |         |             |         |
|---------------------------|------------------------------------|---------|-------------|---------|
|                           | 2 mg/kg bw                         |         | 75 mg/kg bw |         |
|                           | Males                              | Females | Males       | Females |
| <b><i>anti</i> isomer</b> |                                    |         |             |         |
| Bile                      | 38.3                               | 56.1    | 36.5        | 61.1    |
| Faeces                    | 32.5                               | 28.3    | 38.3        | 20.4    |
| Urine                     | 22.1                               | 12.2    | 16.3        | 15.9    |
| Total absorbed            | 60.6                               | 68.6    | 53.0        | 77.2    |
| <b><i>syn</i> isomer</b>  |                                    |         |             |         |
| Bile                      | 56.3                               | 48.9    | 58.0        | 41.6    |
| Faeces                    | 23.8                               | 19.8    | 34.1        | 48.3    |
| Urine                     | 15.4                               | 26.0    | 7.6         | 7.0     |
| Total absorbed            | 71.8                               | 75.1    | 65.7        | 48.7    |

From Shaw (2008a)

dose in males and 48.7% of the dose in females, as calculated from the biliary and urinary excretion and TRR remaining in the carcass.

*Syn* and *anti* isomers at both dose levels in both sexes were excreted at 84.4–97.0% of the administered dose within 24 hours. At 72 hours post-dosing, mean blood and plasma concentrations were below the limit of reliable detection in both sexes in all four groups.

In conclusion, there were no significant differences in absorption and excretion route and rate between the two isomers. Additionally, there was no significant influence of sex or dose (Shaw, 2008a).

To investigate the excretion pattern and tissue distribution of isopyrazam, single doses of 1 mg/kg bw (4.88 MBq/mg) or 75 mg/kg bw (0.08 MBq/mg) of 99.4% radiochemically pure *syn* [pyrazole-5-<sup>14</sup>C]isopyrazam (labelled 98.8:1.2 *syn:anti*; non-labelled 92.8:7.2 *syn:anti*) were administered by oral gavage to four bile duct-cannulated Han Wistar rats of each sex per dose. Urine, bile, faeces and cage washes were collected over 2 days, after which the rats were terminated. A terminal blood sample was removed, together with the gastrointestinal tract and residual carcass, and each sample taken was then analysed for TRR.

At 1 mg/kg bw, the major routes of elimination in both males and females were the bile (means of 57.9% and 47.6% of the administered dose in males and females, respectively) and the faeces (means of 26.4% and 35.7% of the administered dose in males and females, respectively), recovered over 2 days post-dosing. Urinary excretion accounted for means of 14.9% and 15.9% of the dose in males and females, respectively. Absorption accounted for 72.9% of the dose in males and 63.7% of the dose in females, as calculated from the biliary and urinary excretion and TRR remaining in the carcass. At 75 mg/kg bw, the major routes of elimination in both males and females were the bile (means of 54.7% and 57.0% of the administered dose in males and females, respectively) and the faeces (means of 27.3% and 21.2% of the administered dose in males and females, respectively), recovered over 2 days post-dosing. Urinary excretion accounted for means of 7.3% and 13.6% of the dose in males and females, respectively. Absorption accounted for 63.1% of the dose in males and 71.4% of the dose in females, as calculated from the biliary and urinary excretion and TRR remaining in the carcass. Irrespective of dose and sex, 91.0–97.1% of the administered dose was excreted

within 24 hours post-dosing. Mean blood and plasma concentrations were below the limit of reliable detection at 48 hours post-dosing in both dose groups (Shaw, 2008c).

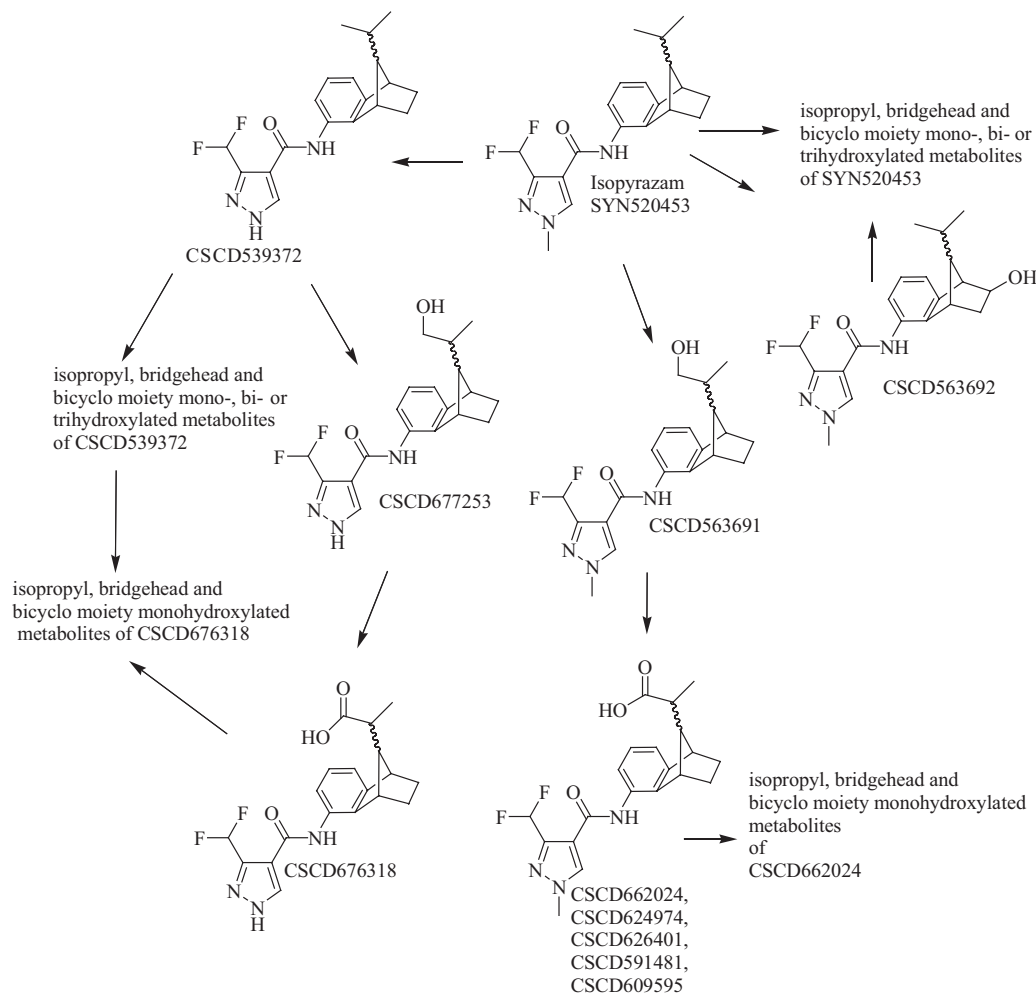
## 1.2 Biotransformation

The metabolism of isopyrazam was investigated by analysing bile, faeces, urine and plasma samples retained from the previous studies: single oral administration of 1 or 75 mg/kg bw of *syn* [pyrazole-5-<sup>14</sup>C]isopyrazam (Allan, 2008a; Shaw, 2008b,c), 14 oral doses of 1 mg/kg bw per day of *syn* [pyrazole-5-<sup>14</sup>C]isopyrazam (Allan, 2008c), single oral administration of 2 or 75 mg/kg bw of *syn* [pyrazole-5-<sup>14</sup>C]isopyrazam and single oral administration of 2 or 75 mg/kg bw of *anti* [pyrazole-5-<sup>14</sup>C]isopyrazam (Shaw, 2008a).

The predominant metabolic pathway for isopyrazam is hydroxylation in the bicyclic isopropyl moiety, followed by further oxidation to form the carboxylic acid or to give rise to multiple hydroxyl moieties, with subsequent formation of glucuronic acid or sulfate conjugates (in Figure 4, the primary metabolism of isopyrazam is depicted; the sulfate and glucuronic acid conjugation products are not shown). Isopyrazam is also oxidized by *N*-demethylation, giving rise to the corresponding set of demethylated metabolites and their conjugates. The structure of isopyrazam provides the potential for stereoisomerization of most metabolites. For many metabolites, it was not possible to determine the exact position of hydroxylation or the conformation of the molecule. No cleavage of the molecule was observed. In general, metabolites excreted in urine tended to be unconjugated, comprising monohydroxy, dihydroxy, acid and hydroxy acids and their demethylated counterparts. In the urine of female rats, sulfate conjugates of these molecules were also detected. In bile, the glucuronide conjugates of the same oxidative metabolites were observed, along with a few metabolites in the unconjugated form. Male rat bile also contained small quantities of trihydroxylated metabolites, present as both conjugated and unconjugated entities. A similar range of metabolites was present in faeces. Some sulfate conjugates were detected in the faeces of female rats. There was less than 1% parent compound detected in the faeces of non-bile duct-cannulated rats. Isopyrazam was the only component detected in the faeces of bile duct-cannulated animals administered a high dose and was the major component in the faeces of bile duct-cannulated animals administered a low dose; the other components were identified as trihydroxy, dihydroxy and *N*-desmethyl dihydroxy metabolites. The metabolic profile of male plasma sampled at the time of the maximum plasma radioactivity concentration ( $T_{\max}$ ) showed the presence of two acid metabolites and three demethylated acid metabolites, with no parent compound present. In female  $T_{\max}$  plasma samples, demethylated parent was the most abundant metabolite, with two *N*-desmethyl hydroxylated metabolites, an *N*-desmethyl dihydroxy metabolite, a sulfate conjugate of the *N*-desmethyl dihydroxy metabolite and an acid metabolite. Some parent compound was also detected. Given the high number of isopyrazam metabolites, each at low concentrations, sex and dosing regimen comparisons for metabolite profiles are difficult. Nevertheless, sex, dosing regimen and isomeric form of isopyrazam do not seem to influence the metabolism of isopyrazam (Green, 2008).

The in vitro metabolism of CSCD658108 (the *syn* isomer of CSCD563692; see Figure 4) and of SYN534969 (the *syn* isomer of SYN520453) by male Wistar rat liver microsomes was investigated. The compounds used were [pyrazole-5-<sup>14</sup>C]CSCD658108 (specific activity 0.89 MBq/mg) and [pyrazole-5-<sup>14</sup>C]SYN534969 (specific activity 2.54 MBq/mg). The compounds were incubated with microsomes at concentrations of 6.316–6.824  $\mu\text{mol/l}$  for 15 minutes, and then metabolites were analysed.

CSCD658108 and SYN534969 were both metabolized to CSCD459488 and further to CSCD656800 (isopropyl and bicyclo dihydroxylated *syn* isopyrazam, also found in vivo in rats). Therefore, CSCD658108 also seems to be an in vivo precursor of CSCD656800 (White, 2008).

**Figure 4. Metabolism of isopyrazam in the rat**

### 1.3 Effects on enzymes and other biochemical parameters

No information was available.

## 2. Toxicological studies

### 2.1 Acute toxicity

The results of studies of acute toxicity with isopyrazam are summarized in [Table 3](#).

#### (a) Oral administration

The acute oral toxicity of *syn* isopyrazam (batch No. SMU6BP001, purity 99.0%), *anti* isopyrazam (batch No. SMU6CP001, purity 95.5%) and isopyrazam (1:1 *syn:anti*, batch No. SMU6CP014, purity 98.2%) was tested in female HanRCC Wistar rats. The median lethal dose ( $LD_{50}$ ) of pure *syn* isopyrazam in female rats was greater than 2000 mg/kg bw; the  $LD_{50}$  of both pure *anti* and 1:1 *syn:anti* isopyrazam was 310.2 mg/kg bw (95% confidence interval [CI] 175–550 mg/kg bw) (Ott, 2006a).

**Table 3. Acute toxicity of isopyrazam**

| Diastereoisomer ratio     | Species | Strain      | Sex          | Route       | LD <sub>50</sub> (mg/kg bw) | LC <sub>50</sub> (mg/l) | Purity (%) | Reference       |
|---------------------------|---------|-------------|--------------|-------------|-----------------------------|-------------------------|------------|-----------------|
| <i>syn</i>                | Rat     | HanRCC:WIST | Female       | Oral        | > 2000                      | —                       | 99.0       | Ott (2006a)     |
| <i>anti</i>               | Rat     | HanRCC:WIST | Female       | Oral        | 310.2 (95% CI 175–550)      | —                       | 95.5       | Ott (2006a)     |
| <i>syn:anti</i> 1:1       | Rat     | HanRCC:WIST | Female       | Oral        | 310.2 (95% CI 175–550)      | —                       | 98.2       | Ott (2006a)     |
| <i>syn:anti</i> 92.8:7.2  | Rat     | HanRCC:WIST | Female       | Oral        | > 2000                      | —                       | 96.4       | Arcelin (2007a) |
| <i>syn:anti</i> 69.7:30.3 | Rat     | HanRCC:WIST | Female       | Oral        | 2000 (95% CI 864.4–4210)    | —                       | 90.8       | Simon (2008c)   |
| <i>syn:anti</i> 92.8:7.2  | Rat     | HanRCC:WIST | Male, female | Dermal      | > 5000                      | —                       | 96.45      | Arcelin (2007b) |
| <i>syn:anti</i> 92.8:7.2  | Rat     | HsdBr/Han   | Male, female | Respiratory | —                           | > 5.28                  | 96.45      | Rattray (2006a) |

CI, confidence interval; LC<sub>50</sub>, median lethal concentration; LD<sub>50</sub>, median lethal dose

The acute oral toxicity of isopyrazam (92.8:7.2 *syn:anti*, batch No. SMU6AP001, purity 96.4%) was tested in female HanRCC Wistar rats. The LD<sub>50</sub> of isopyrazam with a high *syn* epimer fraction in female rats was greater than 2000 mg/kg bw (Arcelin, 2007a).

The acute oral toxicity of isopyrazam (69.7:30.3 *syn:anti*, batch No. SMU7DP017, purity 90.8%) was tested in female HanRCC Wistar rats. The LD<sub>50</sub> of isopyrazam with a two thirds *syn* epimer fraction in female rats was 2000 mg/kg bw (95% CI 864.4–4210 mg/kg bw). Treated animals showed clinical signs (ruffled fur > hunched posture > sedation) a few hours after dosing, which increased in severity with dose. All clinical signs were reversible by 3–8 days post-dosing (Simon, 2008c).

(b) *Dermal application*

The acute dermal toxicity of isopyrazam (92.8:7.2 *syn:anti*, batch No. SMU6AP001, purity 96.4%) was tested in male and female HanRCC Wistar rats. The dermal LD<sub>50</sub> of isopyrazam with a high *syn* epimer fraction in male and female rats was greater than 5000 mg/kg bw (Arcelin, 2007b).

(c) *Exposure by inhalation*

The acute respiratory toxicity of isopyrazam (92.8:7.2 *syn:anti*, batch No. SMU6AP001, purity 96.45%) after a 4-hour nose-only exposure was tested in male and female HsdBr/Han rats. The respiratory median lethal concentration (LC<sub>50</sub>) of isopyrazam with a high *syn* epimer fraction in male and female rats was greater than 5.28 mg/l (Rattray, 2006a).

(d) *Dermal and ocular irritation*

The primary skin irritation potential of 0.5 g isopyrazam per animal (92.8:7.2 *syn:anti*, batch No. SMU6AP001, purity 96.45%) was tested in male and female New Zealand White rabbits following exposure for 4 hours by semi-occlusive dressing. Isopyrazam with a high *syn* epimer fraction was not irritating to the skin in male and female rabbits (Ott, 2006b).

The primary eye irritation potential of 0.1 g isopyrazam per animal (92.8:7.2 *syn:anti*, batch No. SMU6AP001, purity 96.45%) was tested in male and female New Zealand White rabbits by instillation into the left eye of the animals. Isopyrazam with a high *syn* epimer fraction was only

initially slightly irritating to the conjunctivae (reddening and chemosis) in male and female rabbits. There were no other effects on the eye (Talvioja, 2007).

(e) *Dermal sensitization*

In a mouse local lymph node assay to investigate the skin sensitizing potential of isopyrazam, isopyrazam (92.8:7.2 *syn:anti*, batch No. SMU6AP001, purity 96.45%) was tested in female CBA/ca/Ola/hsd mice. Animals were exposed to approximately 25 µl of 0%, 10%, 25% or 60% weight per volume (w/v) isopyrazam in dimethyl sulfoxide (DMSO). A positive response was obtained with 60% w/v isopyrazam. Thus, isopyrazam with a high *syn* epimer fraction exhibited skin sensitizing potential in female mice (Betts, 2006).

## 2.2 *Short-term studies of toxicity*

### *Mice*

Isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 92.8:7.2 *syn:anti*) was administered for 90 days in the diet to groups of 10 male and 10 female 5- to 6-week-old C57BL/10JfCD-1 mice in a range-finding study for a carcinogenicity study. The concentrations in feed were 0, 500, 2500 and 7000 parts per million (ppm) (equal to 0, 76.5, 390.8 and 1382.8 mg/kg bw per day in males and 0, 87.2, 448.9 and 1759.6 mg/kg bw per day in females). Detailed clinical observations, body weights and feed consumption were recorded weekly. In week 12, urine was collected for urinalysis, and a functional observational battery (FOB) was performed. At study termination, haematology, clinical chemistry, organ weights and liver histology from all animals were investigated. Histology in all other organs was performed only in the control and high-dose groups.

Towards the end of the study, body weights in male and female mice were statistically significantly lower by approximately 5–7% at 2500 ppm and by approximately 11% at 7000 ppm. Feed consumption was elevated by approximately 10% in both sexes at 500 and 2500 ppm and by 38% in males and by 54% in females at 7000 ppm. As a result, feed conversion was low at 7000 ppm. At 7000 ppm, females had statistically significantly lower mean haemoglobin concentration, haematocrit and mean cell volume compared with control animals. At 7000 ppm, males had lower red blood cell counts and statistically significantly lower red blood cell distribution width than controls at all dose levels. Platelet counts were statistically significantly increased for all male groups, but without a clear dose–response relationship, and for females at 7000 ppm. In males at 7000 ppm, eosinophils were statistically significantly higher and monocytes were lower than in controls. In females at 7000 ppm, the plasma albumin to globulin ratio was lowered, triglyceride levels were increased by 100% and alanine aminotransferase (ALT) activity was increased by 90%. Body weight–adjusted brain weights were statistically significantly increased by 5% in all dosed males. Body weight–adjusted liver weights were increased dose relatedly in both sexes at all dose levels: by approximately 8% at 500 ppm, 25–32% at 2500 ppm and 47–59% at 7000 ppm. Histological findings were confined to the liver. In all males of all treated groups, hepatocellular hypertrophy was observed, which was slightly more pronounced at 2500 ppm than at 500 ppm and was most pronounced at 7000 ppm. In females, no hepatocellular hypertrophy was observed at 500 ppm, but hepatocellular hypertrophy was evident in 9 of 10 and 10 of 10 animals at 2500 ppm and 7000 ppm, respectively. In one male and one female high-dose animal, necrotic liver nodules were also observed.

The no-observed-adverse-effect level (NOAEL) was 2500 ppm (equal to 390.8 mg/kg bw per day), based on haematological changes at 7000 ppm (equal to 1382.8 mg/kg bw per day) (Pinto, 2008b).

### *Rats*

In a study to compare the toxicity of the *syn* and *anti* conformation isomers of isopyrazam, pure *syn* epimer SYN534969 technical (batch No. SMU6BP001, 99.0% pure), pure *anti* epimer

SYN534968 technical (batch No. SMU6CP001, 95.5% pure) and 1:1 *syn:anti* epimer SYN520453 technical (batch No. SMU6CP014, 98.2% pure) were administered for 28 days in the diet to groups of five male and five female 6- to 7-week-old HsdRCCHan:WIST rats. The concentrations in feed were 0, 500, 2000 and 5000 ppm (equal to 0, 45.2, 176.7 and 437.3 mg/kg bw per day in males and 0, 45.3, 187.4 and 401.0 mg/kg bw per day in females) for all three compounds. Clinical observations and body weight were recorded daily, and feed consumption was recorded weekly. At study termination, haematology and clinical chemistry parameters were analysed and organ weights were recorded. Organs of all main study low-dose and high-dose animals as well as livers from the intermediate-group animals were examined histologically. The 7-ethoxyresorufin *O*-deethylase (EROD, cytochrome P450 [CYP] 1A1/2) and 7-pentoxoresorufin *O*-dealkylase (PROD, CYP2B1/2) activities of liver microsomes were analysed.

In males, there were no clinical signs at any dose level with any compound. With pure *syn* epimer, there were no clinical signs in females. With pure *anti* epimer, one female in the 5000 ppm group showed hunched posture on days 6–8, and two females had piloerection on days 10–26. One female in the 2000 ppm group also had a single observation of piloerection on day 12. With 1:1 *syn:anti*, there were three females with hunched posture at days 4–8 and four females with piloerection on days 4–26. Feed intake was slightly lower than in controls with pure *syn* in high-dose females (Table 4). With pure *anti* and 1:1 *syn:anti*, feed intake was reduced in females at all dose levels and in males at 2000 and 5000 ppm. With pure *anti* and 1:1 *syn:anti*, body weights were reduced at 2000 and 5000 ppm in a dose-dependent manner in both sexes and were also slightly reduced in females with both compounds at 500 ppm. With pure *syn*, the body weights of males and females at 5000 ppm were only slightly reduced, mainly during the 1st week of treatment (Table 5). All three compounds had effects on haematological parameters. With pure *syn*, high-dose males had lower platelet and lymphocyte counts, and high-dose females had lower basophilic granulocytes. With pure *anti*, mid- and high-dose females had lower basophilic granulocytes, prothrombin time was increased in females at 5000 ppm and activated partial prothromboplastin time was lower in all treated females. In females at 5000 ppm, haematocrit and haemoglobin levels were increased; in males at this dose level, the red blood cell counts were elevated. With 1:1 *syn:anti*, high-dose females had elevated red blood cell counts and slightly lower activated partial prothromboplastin time. All three compounds affected blood clinical chemistry parameters (Table 6). With pure *syn*, cholesterol levels were increased in all dosed females, with a statistically significant increase of 40% at 5000 ppm. In males, cholesterol was increased statistically significantly only at the low and middle doses, but not at the high dose. In high-dose males, alkaline phosphatase (ALP) activity was lowered statistically significantly by 30%. With pure *anti*, cholesterol levels were increased in all dosed females, by 40% statistically significantly at 2000 ppm and by 54% statistically significantly at 5000 ppm. Plasma albumin and total protein were decreased by approximately 10% in females at 2000 and 5000 ppm. In females at 5000 ppm, statistically significant increases in potassium by 21% and in phosphorus by 36% were observed. In males, plasma triglycerides were decreased statistically significantly by 35% at 2000 ppm and by 66% at 5000 ppm. Gamma-glutamyltransferase (GGT) activity was increased in male and female high-dose animals by 24%. The activity of ALT in high-dose females was elevated by 43%; the ALP activity in males was elevated by 33%. With 1:1 *syn:anti*, plasma albumin and total protein were decreased by approximately 9% in females at 5000 ppm. Cholesterol levels were increased in all dosed females, by 34% statistically significantly at 2000 ppm and by 63% statistically significantly at 5000 ppm. All dosed females and males at 2000 and 5000 ppm had increased body weight-adjusted liver weights (Table 7). At all dose levels in both sexes for all compounds, the total hepatic P450 content was slightly increased, with no dose dependency (Table 8). Similarly, the EROD activity was elevated in all dosed groups, but not in a compound-, dose- or sex-related manner. PROD activity was increased significantly and dose relatedly, but the increase was not significantly different between sexes and compounds. Hepatocellular hypertrophy was observed in all males in all dose

**Table 4. Feed intake in male and female rats exposed to pure syn, pure anti and 1:1 syn:anti isopyrazam**

|                     | Feed intake (% of control group) |          |          |         |          |          |
|---------------------|----------------------------------|----------|----------|---------|----------|----------|
|                     | Males                            |          |          | Females |          |          |
|                     | 500 ppm                          | 2000 ppm | 5000 ppm | 500 ppm | 2000 ppm | 5000 ppm |
| <b>Pure syn</b>     |                                  |          |          |         |          |          |
| Week 1              | 120                              | 112      | 102      | 100     | 99       | 88       |
| Week 2              | 110                              | 102      | 104      | 103     | 103      | 104      |
| Week 3              | 115                              | 103      | 102      | 101     | 98       | 94       |
| Week 4              | 112                              | 104      | 100      | 97      | 93       | 81       |
| <b>Pure anti</b>    |                                  |          |          |         |          |          |
| Week 1              | 110                              | 92       | 66       | 90      | 69       | 55       |
| Week 2              | 97                               | 91       | 83       | 97      | 87       | 79       |
| Week 3              | 101                              | 93       | 86       | 93      | 107      | 69       |
| Week 4              | 100                              | 90       | 78       | 88      | 75       | 63       |
| <b>1:1 syn:anti</b> |                                  |          |          |         |          |          |
| Week 1              | 96                               | 88       | 78       | 94      | 83       | 50       |
| Week 2              | 99                               | 105      | 100      | 96      | 108      | 78       |
| Week 3              | 103                              | 103      | 96       | 92      | 99       | 69       |
| Week 4              | 102                              | 103      | 91       | 86      | 98       | 62       |

From Milburn (2007e)

**Table 5. Terminal body weight in male and female rats exposed to pure syn, pure anti and 1:1 syn:anti isopyrazam**

|              | Body weight (% of control group) |          |          |         |          |          |
|--------------|----------------------------------|----------|----------|---------|----------|----------|
|              | Males                            |          |          | Females |          |          |
|              | 500 ppm                          | 2000 ppm | 5000 ppm | 500 ppm | 2000 ppm | 5000 ppm |
| Pure syn     | 100                              | 100      | 95       | 100     | 100      | 95*      |
| Pure anti    | 100                              | 89**     | 80**     | 96      | 83**     | 82**     |
| 1:1 syn:anti | 100                              | 94       | 86**     | 97      | 89**     | 79**     |

From Milburn (2007e)

\*  $P < 0.05$ ; \*\*  $P < 0.01$ **Table 6. Blood clinical chemistry parameter changes in male and female rats exposed to pure syn, pure anti and 1:1 syn:anti isopyrazam**

|                  | Parameter changes (% of control group) |          |          |         |          |          |
|------------------|----------------------------------------|----------|----------|---------|----------|----------|
|                  | Males                                  |          |          | Females |          |          |
|                  | 500 ppm                                | 2000 ppm | 5000 ppm | 500 ppm | 2000 ppm | 5000 ppm |
| <b>Pure syn</b>  |                                        |          |          |         |          |          |
| Cholesterol      | +39*                                   | +39*     | +6       | +25     | +13      | +40**    |
| <b>Pure anti</b> |                                        |          |          |         |          |          |
| Cholesterol      | +11                                    | +13      | +11      | +25     | +40**    | +54**    |
| Total protein    | +2                                     | +2       | +4       | +4      | -11**    | -9**     |
| Potassium        | +2                                     | +14      | +5       | +4      | +6       | +21*     |

**Table 6 (continued)**

|                     | Parameter changes (% of control group) |          |          |         |          |          |
|---------------------|----------------------------------------|----------|----------|---------|----------|----------|
|                     | Males                                  |          |          | Females |          |          |
|                     | 500 ppm                                | 2000 ppm | 5000 ppm | 500 ppm | 2000 ppm | 5000 ppm |
| Phosphorus          | -3                                     | +19      | -10      | +18     | +15      | +36*     |
| Triglycerides       | -21                                    | -35*     | -66**    | +42*    | +28      | -16      |
| GGT                 | -2                                     | +23*     | +24*     | -10     | -9       | +24*     |
| ALT                 | -10                                    | +12      | +16      | +29     | +2       | +43*     |
| <b>1:1 syn:anti</b> |                                        |          |          |         |          |          |
| Cholesterol         | +11                                    | 0        | +6       | +23     | +34*     | +63**    |
| Total protein       | -1                                     | -1       | +3       | -2      | 0        | -9*      |

From Milburn (2007e)

ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; \*  $P < 0.05$ ; \*\*  $P < 0.01$ **Table 7. Body weight-adjusted liver weight changes in male and female rats exposed to pure syn, pure anti and 1:1 syn:anti isopyrazam**

|                     | Liver weight changes (% of control group) |          |          |         |          |          |
|---------------------|-------------------------------------------|----------|----------|---------|----------|----------|
|                     | Males                                     |          |          | Females |          |          |
|                     | 500 ppm                                   | 2000 ppm | 5000 ppm | 500 ppm | 2000 ppm | 5000 ppm |
| Pure <i>syn</i>     | —                                         | +17**    | +25**    | +22**   | +31**    | +41**    |
| Pure <i>anti</i>    | —                                         | +20**    | +23**    | +21**   | +20**    | +38**    |
| 1:1 <i>syn:anti</i> | —                                         | +18**    | +30**    | +15**   | +34**    | +25**    |

From Milburn (2007e)

— no effect; \*  $P < 0.05$ ; \*\*  $P < 0.01$ **Table 8. Hepatic P450 content and PROD and EROD activity changes in male and female rats exposed to pure syn, pure anti and 1:1 syn:anti isopyrazam**

|                            | Changes (expressed as ratio to control group values) |          |          |         |          |          |
|----------------------------|------------------------------------------------------|----------|----------|---------|----------|----------|
|                            | Males                                                |          |          | Females |          |          |
|                            | 500 ppm                                              | 2000 ppm | 5000 ppm | 500 ppm | 2000 ppm | 5000 ppm |
| <b>Pure <i>syn</i></b>     |                                                      |          |          |         |          |          |
| P450                       | 1.4*                                                 | 2.1**    | 1.9**    | 1.3     | 1.6*     | 1.4      |
| PROD                       | 88.7**                                               | 133.8**  | 159.7**  | 128.6** | 215.3**  | 180.0**  |
| EROD                       | 2.2**                                                | 3.1**    | 2.9**    | 1.4     | 2.1**    | 1.7**    |
| <b>Pure <i>anti</i></b>    |                                                      |          |          |         |          |          |
| P450                       | 1.1                                                  | 1.6**    | 1.4      | 0.8     | 0.8      | 1.1      |
| PROD                       | 35.7**                                               | 161.6**  | 201.7**  | 42.9**  | 159.7**  | 384.5**  |
| EROD                       | 1.6*                                                 | 2.6**    | 2.2**    | 1.2     | 1.4*     | 2.0**    |
| <b>1:1 <i>syn:anti</i></b> |                                                      |          |          |         |          |          |
| P450                       | 1.6**                                                | 2.2**    | 1.7**    | 1.3     | 1.3      | 2.0**    |
| PROD                       | 77.4**                                               | 164.3**  | 283.4**  | 62.9**  | 181.2**  | 263.2**  |
| EROD                       | 2.3**                                                | 3.6**    | 3.7**    | 1.4     | 1.7**    | 2.1**    |

From Milburn (2007e)

P450, cytochrome P450; EROD, 7-ethoxyresorufin *O*-deethylase; PROD, 7-pentoxyresorufin *O*-dealkylase; \*  $P < 0.05$ ; \*\*  $P < 0.01$

groups with all three compounds, becoming increasingly pronounced (minimal/slight/moderate) with increasing dose. The only exception was with animals receiving 500 or 2000 ppm 1:1 *syn:anti*, where it was equally pronounced. In all female dose groups with all three compounds, the incidence and extent of hepatocellular hypertrophy increased with dose.

Regarding feed consumption, body weight development and haematological and clinical chemistry parameters, females were more sensitive than males for all three compounds. The severity of the effects was highest with pure *anti* isomer, followed by 1:1 *syn:anti* and then by pure *syn* isomer. Liver weight was increased in all dosed female groups and in all male groups at 2000 ppm and above. For liver weight increase, no compound-specific sensitivity was observed. No compound or sex difference was observed for effects on liver histology or on total P450 content or PROD or EROD activity. All compounds mildly elevated total hepatic P450 content and EROD (CYP1A1) activity in both sexes, but markedly increased PROD (CYP2B family) activity. The Meeting considered the liver findings as adaptive effects and not toxicologically relevant.

The NOAEL for pure *syn* was 2000 ppm (equal to 176.7 mg/kg bw per day), based on reduced body weight gain and increased cholesterol levels at 5000 ppm (equal to 437.3 mg/kg bw per day).

The NOAEL for pure *anti* and 1:1 *syn:anti* was 500 ppm (equal to 45.2 mg/kg bw per day), based on reduced body weight gain and increased cholesterol levels at 2000 ppm (equal to 176.7 mg/kg bw per day) (Milburn, 2007e).

Isopyrazam (SYN520453 technical, batch No. TE-5854/7, purity not certified, 89:11 *syn:anti*) was administered for 28 days in the diet to groups of five male and five female 6- to 7-week-old HsdRCCHan:WIST rats. The concentrations in feed were 0, 100, 500 and 2000 ppm (equal to 0, 9.1, 46.1 and 174.9 mg/kg bw per day in males and 0, 9.6, 48.1 and 191.2 mg/kg bw per day in females). Detailed clinical observations and body weight were recorded twice weekly, and feed consumption was recorded weekly. At week 4, control and high-dose animals were subjected to a FOB, and locomotor activity was monitored within the detailed clinical observation. During week 4, urine from control and high-dose animals was collected for urinalysis. At study termination, haematology, clinical chemistry, organ weights and histology (including livers of low- and mid-dose animals) were investigated in control and high-dose animals. The EROD and PROD activities of liver microsomes were analysed.

There were no treatment-related clinical signs or effects in the FOB. In females at 2000 ppm, there was a slight decrease in feed consumption; concomitantly, these females had slightly lower body weights (maximally 7% compared with controls). In males at 2000 ppm, lymphocyte counts were decreased statistically significantly by 30%, and females had a prolonged activated partial thromboplastin time. At 2000 ppm, creatinine levels (+20%) and creatinine kinase activity (+56%) were increased, triglyceride levels were lowered by 39% in males and urea levels were increased by 18%. High-dose males had statistically significantly higher body weight-adjusted brain weights (+4%) and liver weights (+13%). In high-dose females, body weight-adjusted liver weights were increased statistically significantly by 14%. One male in the 500 ppm group and all males and one female in the 2000 ppm group showed centrilobular hypertrophy. At 2000 ppm, total hepatic P450 content was slightly increased in both sexes and EROD activity was moderately increased in males and females. PROD activity was significantly increased at 500 and 2000 ppm in females and at 2000 ppm in males (for 500 ppm males, no data were generated) (Table 9).

The NOAEL was 500 ppm (equal to 46.1 mg/kg bw per day), based on clinical chemistry and haematological changes at 2000 ppm (equal to 174.9 mg/kg bw per day) (Milburn, 2007a).

Isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 92.8:7.2 *syn:anti*) was administered for 28 days in the diet to groups of five male and five female 5- to 6-week-old

**Table 9. Hepatic P450 content and PROD and EROD activity changes in male and female rats exposed to isopyrazam**

|      | Changes (expressed as ratio to control group values) |          |         |          |
|------|------------------------------------------------------|----------|---------|----------|
|      | Males                                                |          | Females |          |
|      | 500 ppm                                              | 2000 ppm | 500 ppm | 2000 ppm |
| P450 | —                                                    | 3.0**    | 1.3     | 1.6*     |
| PROD | —                                                    | 99.1**   | 39.9**  | 74.2**   |
| EROD | —                                                    | 6.8**    | 1.5     | 2.6**    |

From Milburn (2007a)

P450, cytochrome P450; EROD, 7-ethoxyresorufin *O*-deethylase; PROD, 7-pentoxoresorufin *O*-dealkylase; — not measured; \*  $P < 0.05$ ; \*\*  $P < 0.01$

HsdRCCHan:WIST rats. The concentrations in feed were 0, 300, 4000 or 8000 ppm (equal to 0, 29.4, 392.5 and 792.6 mg/kg bw per day in males and 0, 28.1, 390.1 and 720.6 mg/kg bw per day in females). Detailed clinical observations were recorded daily, body weights were measured twice weekly and feed consumption was measured weekly. At study termination, haematology, clinical chemistry, organ weights and liver histology from all animals were investigated.

There were no clinical signs during the study. In the 4000 and 8000 ppm groups, male and female animals had reduced feed consumption and lower body weights compared with control animals. In high-dose males, a slight increase in the prothrombin time was observed. In females, there was a 52% decrease in neutrophil counts at 4000 ppm and a 36% decrease at 8000 ppm, and there was a 34% decrease in eosinophils at 8000 ppm. Several clinical chemistry parameter changes were observed in females at 4000 and 8000 ppm and above (Table 10). In males, triglyceride levels were decreased at all dose levels; at 300 ppm, the effect was not statistically significant. Body weight-adjusted liver weights were increased in both sexes by 22–24% at 4000 ppm and by 28–31% at 8000 ppm. Body weight-adjusted testes weights in 8000 ppm males were increased by 14%. All animals in the 4000 and 8000 ppm groups showed slight to minimal hepatocellular hypertrophy.

The NOAEL was 300 ppm (equal to 28.1 mg/kg bw per day), based on reduced body weight gain and clinical chemistry and haematological changes at 4000 ppm (equal to 390.1 mg/kg bw per day) (Milburn, 2007b).

Isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 92.8:7.2 *syn:anti*) was administered for 90 days in the diet to groups of 12 male and 12 female 5- to 6-week-old HsdRCCHan:WIST rats. The concentrations in feed were 0, 300, 1500 and 6000 ppm (equal to 0, 21.3, 106.3 and 463.0 mg/kg bw per day in males and 0, 23.8, 117.8 and 484.4 mg/kg bw per day in females). The eyes of all animals were examined pre-study, and the eyes of the control and high-dose animals were examined during the week prior to termination. Detailed clinical observations, body weights and feed consumption were recorded weekly. In week 12, urine was collected for urinalysis, and a FOB was performed. At study termination, haematology, clinical chemistry, organ weights and liver histology from all animals were investigated. Histology in all other organs was performed only in the control and high-dose groups.

Feed consumption in females was reduced maximally by 12% at 1500 ppm and by 23% at 6000 ppm. The body weights were decreased in these groups by 7% and 19%, respectively. In males at 6000 ppm, feed consumption was reduced by 18% in the 1st week, and body weight was reduced by maximally 8% only in weeks 2 and 3. There were no treatment-related changes in urinalysis and no treatment-related haematological changes. Neutrophil counts were reduced in 300 ppm males by 23%, at 1500 ppm by 22% and at 6000 ppm by 18%. Statistical significance was attained at the low and middle doses. Eosinophil counts were statistically significantly reduced by 29% in high-dose

**Table 10. Blood clinical chemistry parameter changes in male and female rats exposed to isopyrazam**

|               | Changes (% of control group) |          |          |         |          |          |
|---------------|------------------------------|----------|----------|---------|----------|----------|
|               | Males                        |          |          | Females |          |          |
|               | 300 ppm                      | 4000 ppm | 8000 ppm | 300 ppm | 4000 ppm | 8000 ppm |
| Cholesterol   | -18                          | +2       | -15      | +5      | +33**    | +51**    |
| Creatinine    | -8                           | -5       | -10*     | 0       | -2       | 0        |
| Phosphorus    | +10                          | +7       | +10      | 0       | +24**    | +44**    |
| Triglycerides | -26                          | -54**    | -53**    | +9      | +16      | -4       |
| Urea          | -11                          | -7       | -8       | -7      | +20*     | +34**    |
| GGT           | +1                           | +9       | +21      | +7      | +13      | +45**    |

From Milburn (2007b)

GGT, gamma-glutamyltransferase; \*  $P < 0.05$ ; \*\*  $P < 0.01$

males. In males, triglycerides and bilirubin were decreased statistically significantly at 1500 and 6000 ppm, and cholesterol was increased dose relatedly, but not statistically significantly (Table 11). At the high dose level, males also showed minimally but statistically significantly elevated levels of sodium, chloride and phosphorus and increased activities of GGT, ALT, aspartate aminotransferase (AST) and creatine kinase. In females, bilirubin was decreased statistically significantly at all dose levels, and GGT activity was increased at 1500 ppm and above. High-dose females also showed minimally but statistically significantly elevated levels of sodium and chloride and increased activities of ALT. In high-dose females, cholesterol was increased statistically significantly. Body weight-adjusted liver weights were increased in females at 300 and 1500 ppm by 14% and at 6000 ppm by 19%. In males, liver weights were increased by 14% at 1500 ppm and by 24% at 6000 ppm. Body weight-adjusted heart weights were statistically significantly decreased in males at 1500 ppm and in both sexes at 6000 ppm. In high-dose females, kidney weights were decreased. In males, thymus weights were decreased statistically significantly by 15% at 300 ppm, by 16% at 1500 ppm and by 23% at 6000 ppm. In other rat feeding studies, thymus weights were not affected. Therefore, the effect here was judged not to be treatment related. At the middle and high dose levels, all male and female animals showed minimal to slight hepatocellular hypertrophy.

The NOAEL was 300 ppm (equal to 21.3 mg/kg bw per day), based on clinical chemistry changes at 1500 ppm (equal to 106.3 mg/kg bw per day) (Milburn, 2007c).

Isopyrazam (SYN520453, batch No. KI-7280/6, purity 99.8%, 97.7:2.3 *syn:anti*) was administered for 90 days in the diet to groups of 12 male and 12 female 5- to 6-week-old HsdRCCHan:WIST rats. The concentrations in feed were 0, 250, 1000 and 4000 ppm (equal to 0, 18.9, 74.1 and 314.8 mg/kg bw per day in males and 0, 21.5, 84.8 and 340.7 mg/kg bw per day in females). The eyes of all animals were examined pre-study and prior to termination. Detailed clinical observations, body weights and feed consumption were recorded weekly. In week 12, urine was collected for urinalysis, and a FOB was performed. At study termination, haematology, clinical chemistry, organ weights and liver histology from all animals were investigated. Histology in all other organs was performed only in the control and high-dose groups.

There were no deaths and no compound-related clinical changes, no compound-related effects on the eyes and no compound-related effects on the FOB parameters measured or on motor activity. There were no compound-related effects on haematological or urine clinical chemistry parameters. Males at 4000 ppm had statistically significantly higher platelet counts than controls. Female plasma total protein and plasma calcium were higher in animals at 1000 ppm than in controls. Liver weights

**Table 11. Blood clinical chemistry parameter changes in male and female rats exposed to isopyrazam**

|              | Changes (% of control group) |          |          |         |          |          |
|--------------|------------------------------|----------|----------|---------|----------|----------|
|              | Males                        |          |          | Females |          |          |
|              | 300 ppm                      | 1500 ppm | 6000 ppm | 300 ppm | 1500 ppm | 6000 ppm |
| Cholesterol  | +6                           | +15      | +19      | 0       | +14      | +27**    |
| Triglyceride | -4                           | -28**    | -45**    | -3      | -9       | -16      |
| GGT          | +4                           | +4       | +22**    | +8      | +14*     | +30**    |
| ALT          | +2                           | +10      | +23*     | -7      | +9       | +29*     |

From Milburn (2007c)

ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; \*  $P < 0.05$ ; \*\*  $P < 0.01$

in males and females at 1000 or 4000 ppm were statistically significantly higher than those in controls after adjustment for terminal body weight and showed a dose–response relationship (12% in both sexes at 1000 ppm and 23% and 31% in females and males, respectively, at 4000 ppm). Brain weights in females were higher (3–6%) than those of controls after adjustment for terminal body weight in all dosed animals, statistically significantly at 250 and 4000 ppm. Heart weights in females at 4000 ppm were statistically significantly lower (8%) than controls. Compound-related findings were confined to the liver and consisted of hypertrophy of centrilobular hepatocytes in 10 of 12 males and 8 of 12 females at 1000 ppm and in all animals at 4000 ppm.

The Meeting considered the slightly higher liver weights and hepatocellular hypertrophy in males and females at 1000 ppm as adaptive changes and of no toxicological relevance. The NOAEL was 1000 ppm (equal to 74.1 mg/kg bw per day), based on significantly increased relative liver weights and hepatocellular hypertrophy in males and females at 4000 ppm (equal to 314.8 mg/kg bw per day) (Rattray, 2006b).

In a comparative study, high *syn* isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 92.8:7.2 *syn:anti*) or low *syn* isopyrazam (SYN520453, batch No. SMU7DP017, purity 90.8%, 69.7:30.3 *syn:anti*) was administered for 90 days in the diet to groups of 10 male and 10 female 6-week-old Crl:WI(Han) rats. The concentrations in feed were 0, 100, 250 and 2000 ppm (equal to 0, 8.3, 20.5 and 161.0 mg/kg bw per day in males and 0, 9.7, 24.1 and 195.0 mg/kg bw per day in females). The eyes of all animals were examined pre-study, and the eyes of the control and high-dose animals were examined during the week prior to termination. Detailed clinical observations, body weights and feed consumption were recorded weekly. In week 12, urine was collected for urinalysis, and a FOB was performed. At study termination, haematology, clinical chemistry, organ weights and liver histology from all animals were investigated. Histology of all other organs was performed only in the control and high-dose groups.

In males treated with low *syn* at 2000 ppm, a statistically significant increase in the incidence of animals with piloerection was observed. At 2000 ppm with both compounds in both sexes, body weight gain was slightly lower than in controls, without any obvious difference between the two compounds. The effect was more pronounced in females. Females in the 2000 ppm groups with both compounds had statistically significantly lower ALP activities. Females treated with high *syn* at 2000 ppm had statistically significantly higher cholesterol levels. In 2000 ppm males, thyroid weights were increased compared with controls, statistical significance being attained only in the high *syn* group. Liver weights in high-dose animals of both sexes were increased with both compounds, and these animals also showed centrilobular hypertrophy and midzonal hepatocyte vacuolation.

There was no obvious difference in toxicity between the two compounds.

The NOAEL for both compounds was 250 ppm (equal to 20.5 mg/kg bw per day), based on body weight effects and effects on the liver at 2000 ppm (equal to 161.0 mg/kg bw per day) (Shearer, 2009).

### *Dogs*

Isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 92.8:7.2 *syn:anti*) was administered by gelatine capsule for 90 days to four male and four female 27- to 29-week-old Beagle dogs. The dose levels were 0, 30, 100 and 300 mg/kg bw per day. All dogs were given a full clinical examination by a veterinarian pre-study and prior to termination. Detailed clinical observations and body weights were recorded weekly. Blood samples were taken from all dogs in weeks -2, 4, 8 and 13, and urine samples at weeks -2, 6 and 13. At study termination, organ weights were recorded, and histopathological examinations of all organs were performed.

On day 2 after dosing, two males at 300 mg/kg bw per day showed ataxia and/or inability to stand, slow, jerky forward/backward movements/tremors and absence of some forelimb reflexes. Aggressiveness and excitability with twitching were also observed in one of these animals, and subdued behaviour and increased salivation in another. A third male had reduced activity and slow, side-to-side head movements on day 2 after dosing. One male at 100 mg/kg bw per day showed intermittent, side-to-side head shake/wobble after dosing in week 2 and in week 4. From the start of the study in males and from week 5 in females, body weights were lower by 10–12% in both sexes at 300 mg/kg bw per day. Feed consumption was generally lower in males at 300 mg/kg bw per day throughout the study. Platelet counts in males at 300 mg/kg bw per day were increased compared with controls. Plasma total protein and albumin levels were decreased statistically significantly in both sexes at 300 mg/kg bw per day, in males at study termination and in females from week 4 onwards. Cholesterol levels were dose-relatedly decreased at all dose levels in males and females, although not statistically significantly. ALP activity was increased in both sexes at 100 mg/kg bw per day and above. Urine specific gravity was lower in females at 300 mg/kg bw per day at week 13. Liver weights were increased in males at 100 mg/kg bw per day and in males and females at 300 mg/kg bw per day. In females at all dose levels, ovary, spleen and uterus weights were lowered. In the control group, individual animals showed unusually high weights for these organs, and therefore the decrease in the treated groups seems not to be treatment related.

The NOAEL in this study was 30 mg/kg bw per day, based on behavioural changes and liver weight increases at 100 mg/kg bw per day (Brammer, 2007).

Isopyrazam (SYN520453, batch No. SMU7DP017, purity 90.8%, 69.7:30.3 *syn:anti*) was administered by gelatine capsule for 90 days to four male and four female 25- to 27-week-old Beagle dogs. The dose levels were 0, 10, 30 and 250 mg/kg bw per day. All dogs were given a full clinical (including ophthalmoscopy) examination by a veterinarian pre-study and prior to termination. Detailed clinical observations and body weights were recorded weekly. Blood samples were taken from all dogs in weeks -2, 4, 8 and 13, and urine samples in weeks -2, 6 and 13. At study termination, organ weights were recorded, and histopathological examinations of all organs were performed.

In males and females at 250 mg/kg bw per day, salivation was observed during dosing. A low incidence of abnormal behaviours was seen in one dog at this dose level, which comprised sedation, sporadic buckling of the hindlimbs, uncoordinated movements, shaking the head and ptosis. In addition, decreased activity was recorded once in a different male of this dose group. Loose and watery faeces occurred in both males and females at a higher incidence than in controls. Feed consumption in high-dose animals was reduced initially, but recovered throughout the study. One male and one female dog lost weight during the 1st week of treatment.

The NOAEL in this study was 30 mg/kg bw per day, based on clinical observations and initial body weight loss at 250 mg/kg bw per day (Jackson, 2008a).

Isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 92.8:7.2 *syn:anti*) was administered by gelatine capsule for 52 weeks to four male and four female 28- to 30-week-old Beagle dogs. The dose levels were 0, 25, 100 and 250 mg/kg bw per day. All dogs were given a full clinical (including ophthalmoscopy) examination by a veterinarian pre-study and prior to termination. Detailed clinical observations (by mistake not done at weeks 1, 4, 9, 10, 12, 15, 17, 24, 26, 30, 36, 37, 41, 46 and 49) and body weights were recorded weekly. Blood samples were taken from all dogs pre-study and in weeks 13, 26 and 52, and urine samples were taken pre-test and in weeks –26 and 52. At study termination, organ weights were recorded, and histopathological examinations of all organs were performed.

There were no treatment-related clinical signs. A decrease in mean feed consumption compared with controls was recorded at 250 mg/kg bw per day during the first 6 weeks of treatment. Feed intake subsequently improved in these animals to levels similar to pre-test, although they remained marginally lower than those of the control animals until approximately week 26. Body weight loss was recorded in three males (7.7%, 3.0% and 7.1% reduction) and two females (6.4% and 4.1% reduction) treated at 250 mg/kg bw per day during week 1 of treatment. There was a statistically significant lower body weight gain in males at 250 mg/kg bw per day and occasional similar statistically significant effects in females. At 100 mg/kg bw per day in males, body weight gain was statistically significantly decreased up to day 85. Plasma albumin in males was statistically significantly, and dose relatedly, lowered at all dose levels in week 13 and thereafter only at 100 and 250 mg/kg bw per day. In females, albumin was lowered at 250 mg/kg bw per day. Bilirubin was lowered in males at 250 mg/kg bw per day in week 13 and at 100 and 250 mg/kg bw per day in week 52. ALP activity was increased at all time points at all dose levels in males, attaining statistical significance at week 13 in the 250 mg/kg bw per day group and only in week 52 (2.2-fold increase) at 25 mg/kg bw per day. ALP activity was increased in females at all dose levels, being statistically significant in week 26 in the 250 mg/kg bw per day group and at week 52 in the 100 mg/kg bw per day group. In high-dose males, ALT and glutamate dehydrogenase activities were increased. In females at 250 mg/kg bw per day, body weight-adjusted heart weights were increased by 19%. In males, liver weights were increased at all dose levels, becoming statistically significant (+42% and +53%, respectively) at 100 and 250 mg/kg bw per day. There were no treatment-related histological changes.

The NOAEL in this study was 25 mg/kg bw per day, based on changes in clinical chemistry and in liver weight at higher dose levels. The occasional modest changes in some clinical chemistry parameters at 25 mg/kg bw per day, in the absence of any corresponding histopathological or other changes, were considered not to be of toxicological significance (Jackson, 2008b).

The overall NOAEL for effects of isopyrazam with a *syn:anti* ratio down to 69.7:30.3 in the 3-month and 1-year studies in dogs was 30 mg/kg bw per day.

### 2.3 Long-term studies of toxicity and carcinogenicity

#### *Mice*

Isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 92.8:7.2 *syn:anti*) was administered for 80 weeks in the diet to groups of 50 male and 50 female 5- to 6-week-old C57BL/10J<sub>CD-1</sub> mice. The concentrations in feed were 0, 70, 500 and 3500 ppm (equal to 0, 7.8, 56.2 and 432.6 mg/kg bw per day in males and 0, 9.9, 74.9 and 553.6 mg/kg bw per day in females). The eyes of all main study animals were examined pre-study, at week 50 and prior to termination. Detailed clinical observations, body weights and feed consumption were recorded weekly up to week 15 and then every 2 weeks. A FOB was performed on all interim-kill animals. For haematology, blood was taken from all animals at week 53 and at study termination. At study termination, organs were weighed and examined histologically.

A high number of males in the 3500 ppm group (36/50 versus 5/50, 4/50 and 6/50 in the control, 70 ppm and 500 ppm groups, respectively) showed eye discharge at several time points. Body weight gain was reduced in male and female mice at 3500 ppm (Figure 5). In female mice at 70 ppm, the body weights were higher than in controls. In females at 3500 ppm, lymphocyte counts and eosinophil counts were increased by 24% and 43%, respectively. In males at 3500 ppm, body weight-adjusted spleen weights were statistically significantly reduced by 53% and were still statistically significantly reduced by 17% after exclusion of animals with malignant lymphoma and extramedullary haematopoiesis. In this exclusion group, high-dose females also showed statistically significantly reduced spleen weights (−19%). In females at 3500 ppm, kidney weights were statistically significantly reduced by 9%. In both sexes, liver weights were increased statistically significantly by 13% in the 500 ppm female group and by 38% in the 3500 ppm female group. In males at 3500 ppm, liver weights were increased by 24%.

In the liver, the incidence of hepatocellular hypertrophy was increased at 500 ppm and above in females and at 3500 ppm in males. At 3500 ppm, the incidences of epithelial eosinophilic droplets in the nasal cavity of males and of epithelial eosinophilic droplets in the gall bladder of females were elevated (Table 12).

The incidences of benign and malignant tumours were not increased at any dose level.

The NOAEL was 70 ppm (equal to 9.9 mg/kg bw per day), based on periportal hepatocellular hypertrophy in females at 500 ppm (equal to 74.9 mg/kg bw per day) (Pinto, 2008a).

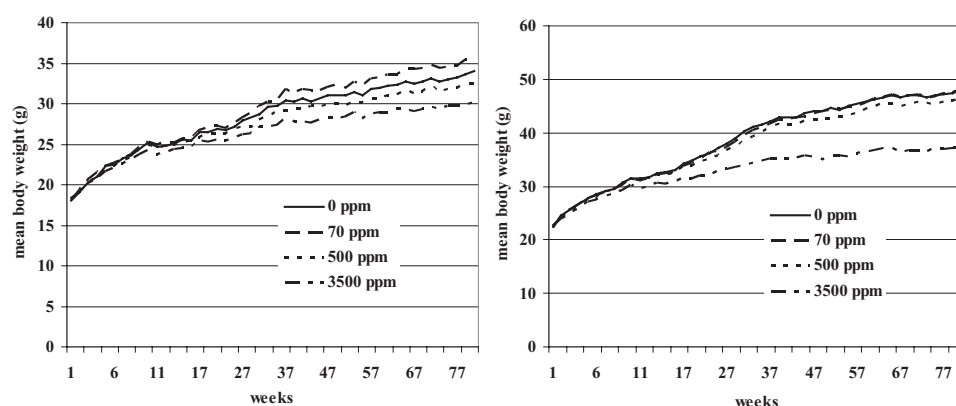
#### *Rats*

Isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 92.8:7.2 *syn:anti*) was administered for 104 weeks in the diet to groups of 64 male and 64 female 5- to 6-week-old HsdRCCHan:WIST rats. Twelve animals of each sex per dose were investigated at an interim kill at 52 weeks. The concentrations in feed were 0, 100, 500 and 3000 ppm (equal to 0, 5.5, 27.6 and 173.5 mg/kg bw per day in males and 0, 6.9, 34.9 and 232.8 mg/kg bw per day in females). The eyes of all main study animals were examined pre-experimentally, at week 50 and prior to termination. Detailed clinical observations and body weights were recorded weekly up to week 15 and then every 2 weeks. Feed consumption was recorded weekly for the first 13 weeks, then in week 16 and thereafter every 4th week. A FOB of tests and locomotor activity monitoring were performed during week 50 on the interim-kill animals. For haematology, blood was taken from 13 animals of each sex per dose at weeks 14, 27, 53 and 79. From the same animals, urine was collected at weeks 13, 26, 52, 78 and 104. For clinical chemistry, blood was taken from another 13 animals of each sex per dose at weeks 14, 27, 53 and 79. At study termination, organs were weighed and examined histologically.

Seventy-five per cent of males and 67% of females survived until study termination. The finding of “small linear opacity in cornea” was increased in 3000 ppm females (5/36 versus 1/40 in the control group). In males, body weight was lower in the 3000 ppm group by maximally 14% (Figure 6). In females, body weight was statistically significantly lower by maximally 30% at 3000 ppm, by 12% at 500 ppm and by maximally 7% at 100 ppm. Body weight gain was reduced in females by 40% at the high dose, by 16% at the middle dose and by 8% at the low dose. In both sexes, feed consumption at 3000 ppm was reduced. In the FOB, hindlimb grip strength was reduced in high-dose females, and these animals had increased motor activity.

In females, there was a dose-related decrease in haemoglobin, haematocrit, and red blood cell and platelet counts, being statistically significant at 3000 ppm at several time points, predominantly in the second part of the study. In males at 3000 ppm, lymphocyte counts were lowered at all time points, with statistical significance at week 53. In these males, monocyte counts were decreased in the second half of the study, the effect becoming statistically significant at the end of the study. Monocyte counts were also low in high-dose females. At the end of the study, but not at interim kill, the prothrombin time was statistically significantly reduced in males by 3% at 100 ppm, by

**Figure 5. Body weights in female (left) and male (right) mice fed isopyrazam in the diet**



Source: Pinto (2008a)

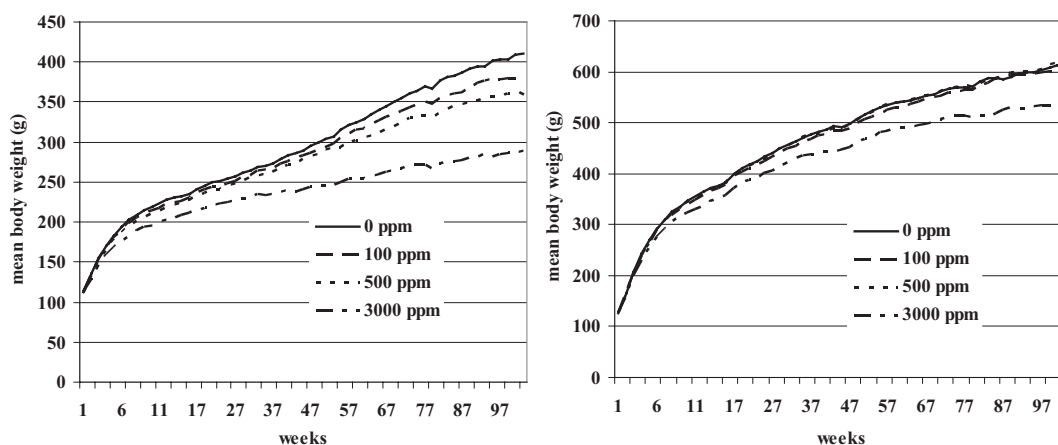
**Table 12. Incidences of non-neoplastic histological changes in male and female mice (terminal kill and preterm deaths combined)**

|                                                | Severity | Incidence of histological finding |       |        |         |         |       |         |         |
|------------------------------------------------|----------|-----------------------------------|-------|--------|---------|---------|-------|---------|---------|
|                                                |          | Males                             |       |        |         | Females |       |         |         |
|                                                |          | Dietary concentration (ppm)       |       |        |         |         |       |         |         |
|                                                |          | 0                                 | 70    | 500    | 3500    | 0       | 70    | 500     | 3500    |
| Hepatocellular hypertrophy                     |          |                                   |       |        |         |         |       |         |         |
| - midzonal                                     | Minimal  | 0                                 | 0     | 5      | 28      | —       | —     | —       | —       |
|                                                | Slight   | 0                                 | 0     | 0      | 12      | —       | —     | —       | —       |
|                                                | Total    | 0/50                              | 0/50  | 5/50   | 40/50** | —       | —     | —       | —       |
| - periportal                                   | Minimal  | 0                                 | 0     | 0      | 2       | 0       | 0     | 13      | 8       |
|                                                | Slight   | 0                                 | 0     | 0      | 0       | 0       | 0     | 0       | 35      |
|                                                | Moderate | 0                                 | 0     | 0      | 0       | 0       | 0     | 0       | 4       |
|                                                | Total    | 0/50                              | 0/50  | 0/50   | 2/50    | 0/50    | 0/50  | 13/50** | 47/50** |
| Nasal cavity/pharynx                           |          |                                   |       |        |         |         |       |         |         |
| - epithelial eosinophilic droplets             | Minimal  | 35                                | 28    | 21     | 29      | 19      | 28    | 23      | 32      |
|                                                | Slight   | 3                                 | 6     | 4      | 1       | 2       | 2     | 2       | 4       |
|                                                | Total    | 38/50                             | 34/50 | 25/50* | 30/50   | 21/50   | 30/50 | 25/50   | 36/50** |
| - inflammation/exudate, nasolacrimal duct      | Minimal  | 3                                 | 9     | 11     | 21      | 5       | 2     | 4       | 6       |
|                                                | Slight   | 2                                 | 0     | 1      | 13      | 0       | 1     | 0       | 0       |
|                                                | Moderate | 1                                 | 0     | 1      | 4       | 0       | 1     | 0       | 0       |
|                                                | Total    | 6/50                              | 9/50  | 13/50  | 38/50** | 5/50    | 4/50  | 4/50    | 6/50    |
| Gall bladder epithelium, eosinophilic droplets | Minimal  | 10                                | 3     | 11     | 11      | 9       | 10    | 8       | 18      |
|                                                | Slight   | 3                                 | 3     | 0      | 2       | 1       | 0     | 5       | 3       |
|                                                | Moderate | 1                                 | 1     | 0      | 1       | 4       | 0     | 1       | 4       |
|                                                | Marked   | 0                                 | 0     | 0      | 0       | 0       | 1     | 1       | 0       |
|                                                | Severe   | 0                                 | 0     | 1      | 1       | 0       | 0     | 0       | 0       |
|                                                | Total    | 14/48                             | 7/44  | 12/48  | 15/49   | 14/48   | 11/49 | 15/50   | 25/50** |

From Pinto (2008a)

— not investigated; \*  $P < 0.05$ ; \*\*  $P < 0.01$

**Figure 6. Body weights in female (left) and male (right) rats fed isopyrazam in the diet**



Source: Milburn (2008)

6% at 500 ppm and by 7% at 3000 ppm. A similar, dose-related effect was observed in females, becoming statistically significant at 3000 ppm. In 500 and 3000 ppm females, the activated partial thromboplastin time was prolonged statistically significantly by 8% at the interim kill and by 4–5% at the end of the study.

Plasma urea was increased (statistically significantly at weeks 53 and 105) by maximally 22% in females at 3000 ppm. In these females, urea was increased at all dose levels, statistically significantly at 100 and 3000 ppm. Plasma creatinine level was elevated statistically significantly at weeks 53 and 79, but not at week 105. Females at 3000 ppm had increased albumin and total protein levels at all time points in the second half of the study, being statistically significant at the end of the study. Cholesterol levels in high-dose females were increased at all time points except at week 105, being statistically significant at weeks 14, 53 and 79. Triglyceride levels in high-dose males were decreased at 500 ppm, statistically significantly at week 27, and at all time points in the 3000 ppm group. In females, triglyceride levels were decreased at all dose levels, statistically significantly at weeks 27, 53 and 79 in the 100 ppm group and at weeks 53, 79 and 105 in the 500 and 3000 ppm groups. In females, bilirubin was dose-relatedly decreased in all dose groups, being statistically significant at week 79 in the 100 ppm group and at most time points in the 500 and 3000 ppm groups. In males and females at 500 and 3000 ppm, ALP activity was decreased statistically significantly at several time points. GGT activity was statistically significantly increased in males at 500 and 3000 ppm in the second half of the study; in females, there was no clear dose-response relationship. ALT activity was increased in high-dose males. AST activity was lowered statistically significantly at week 105 in females at 500 ppm and in the second half of the study at 3000 ppm (Table 13). Sodium and chloride levels were statistically significantly elevated in the 3000 ppm females at most time points. There were no meaningful observations in urinalysis.

At the interim kill at week 52, body weight-adjusted adrenal weights in females in the 3000 ppm group were decreased statistically significantly by 18%. Relative brain weights were increased by 4% in males and decreased by 10% in females. Relative liver weights in both sexes at 3000 ppm were statistically significantly increased by 17% in males and by 14% in females. At terminal kill, relative brain weights in females at 3000 ppm were increased by 4%. Relative liver weights were increased in both sexes, by 5% in males and by 12% in females at 500 ppm and by 17% in males and by 26% in females at 3000 ppm. Absolute and relative adrenal weights in females were lower by 16% at 3000 ppm.

**Table 13. Changes in clinical chemistry parameters in rats administered isopyrazam**

|               | Week | % change compared with control |         |          |         |         |          |
|---------------|------|--------------------------------|---------|----------|---------|---------|----------|
|               |      | Males                          |         |          | Females |         |          |
|               |      | 100 ppm                        | 500 ppm | 3000 ppm | 100 ppm | 500 ppm | 3000 ppm |
| Urea          | 53   | 2                              | -2      | 2        | 0       | 0       | 9*       |
|               | 104  | 0                              | -4      | -16      | 11*     | 9       | 22**     |
| Albumin       | 53   | 1                              | 0       | -2**     | 1       | -2      | 1        |
|               | 104  | -1                             | 1       | 1        | -2      | -1      | 3**      |
| Protein       | 53   | 2                              | 1       | 0        | 0       | -1      | 1        |
|               | 104  | -1                             | 2       | 0        | -2      | 0       | 3*       |
| Cholesterol   | 53   | 7                              | 15      | 15       | 9       | 8       | 31*      |
|               | 104  | 9                              | 24**    | 7        | -1      | -2      | 0        |
| Triglycerides | 53   | 8                              | -18     | -29*     | -20*    | -25*    | -39**    |
|               | 104  | -9                             | -14     | -37**    | 1       | -25**   | -36**    |
| Bilirubin     | 53   | -1                             | 12      | -6       | -2      | -20*    | -11      |
|               | 104  | 9                              | -11     | -11      | -7      | -29**   | -30**    |
| ALP           | 53   | 5                              | -8      | -34**    | -3      | -24*    | -23*     |
|               | 104  | 3                              | -17**   | -34**    | 5       | 7       | -31*     |
| GGT           | 53   | 13                             | 18**    | 25**     | 1       | 14      | 4        |
|               | 104  | 11                             | 25**    | 50**     | 7       | 10      | 11**     |
| ALT           | 53   | -1                             | 2       | 65*      | 26      | 9       | -1       |
|               | 104  | 31                             | 1       | 56*      | -11     | -21*    | -7       |
| AST           | 53   | -5                             | 1       | 41       | 54      | 6       | -23      |
|               | 104  | 6                              | -10     | 2        | -3      | -22*    | -21*     |

From Milburn (2008)

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase;

\*  $P < 0.05$ ; \*\*  $P < 0.01$

Histologically, statistically significantly higher incidences of hepatocellular hypertrophy were observed at all dose levels in both sexes at the terminal kill and at 500 ppm in females at the interim kill (Tables 14 and 15). Statistically significantly increased incidences of hepatocellular pigmentation in females were observed at all dose levels at the end of the study and in high-dose females at the interim kill. Males at the interim kill and at study termination were affected statistically significantly only at the high dose level. Incidences of bile duct hyperplasia or fibrosis were increased statistically significantly in males at all dose levels at the study termination. In a contemporary control study conducted in the same laboratory (PR1359), the incidence of minimal bile duct hyperplasia at 24 months was 6 of 52 in males and 8 of 52 in females. The incidence of epithelial mineralization of the pelvis was decreased statistically significantly in high-dose animals. The incidences of foci of eosinophilic hepatocytes were increased statistically significantly at 500 ppm and above in both sexes at study termination.

In females at 3000 ppm, a statistically significant number of hepatocellular adenomas (17%) was observed (Table 16). At 3000 ppm, one hepatocellular carcinoma was found in each sex. Again in the high-dose females, the incidence (23%) of uterine endometrial adenocarcinoma was statistically significantly increased. According to the sponsor, uterine endometrial adenocarcinoma may occur at up to 7.8% in three in-house control groups, and incidences up to 28% are reported in the Registry of Industrial Toxicology Animal-data (RITA) database.

**Table 14. Incidences of non-neoplastic histological changes in male and female rats at interim kill**

| Severity                                   |         | Incidence                   |           |           |           |           |           |           |           |
|--------------------------------------------|---------|-----------------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
|                                            |         | Males                       |           |           |           | Females   |           |           |           |
|                                            |         |                             |           |           |           |           |           |           |           |
|                                            |         | Dietary concentration (ppm) |           |           |           |           |           |           |           |
|                                            |         | 0                           | 100       | 500       | 3000      | 0         | 100       | 500       | 3000      |
| <i>No. of animals investigated</i>         |         | <i>12</i>                   | <i>12</i> | <i>12</i> | <i>12</i> | <i>12</i> | <i>12</i> | <i>12</i> | <i>12</i> |
| Hepatocellular hypertrophy                 | Minimal | 0                           | 0         | 4         | 5         | 0         | 0         | 9         | 0         |
|                                            | Slight  | 0                           | 0         | 0         | 7         | 0         | 0         | 0         | 12        |
|                                            | Total   | 0                           | 0         | 4         | 7**       | 0         | 0         | 9**       | 12**      |
| Pigmentation hepatocytes/<br>Kupffer cells | Minimal | 0                           | 0         | 0         | 5         | 3         | 4         | 4         | 10        |
|                                            | Slight  | 0                           | 0         | 0         | 1         | 0         | 1         | 0         | 0         |
|                                            | Total   | 0                           | 0         | 0         | 6*        | 3         | 5         | 4         | 10*       |

From Milburn (2008)

\*  $P < 0.05$ ; \*\*  $P < 0.01$ **Table 15. Incidences of non-neoplastic histological changes in male and female rats (terminal kill and preterm deaths combined)**

|                                           |          | Severity                    | Incidence |      |      |         |      |      |      |    |
|-------------------------------------------|----------|-----------------------------|-----------|------|------|---------|------|------|------|----|
|                                           |          | Males                       |           |      |      | Females |      |      |      |    |
|                                           |          | Dietary concentration (ppm) |           |      |      |         |      |      |      |    |
|                                           |          | 0                           | 100       | 500  | 3000 | 0       | 100  | 500  | 3000 |    |
| <i>No. of animals investigated</i>        |          |                             | 52        | 52   | 52   | 52      | 52   | 52   | 52   | 52 |
| Hepatocellular hypertrophy, centrilobular | Minimal  | 0                           | 12        | 23   | 4    | 0       | 22   | 13   | 1    |    |
|                                           | Slight   | 0                           | 0         | 20   | 22   | 0       | 0    | 35   | 13   |    |
|                                           | Moderate | 0                           | 0         | 2    | 23   | 0       | 0    | 1    | 36   |    |
|                                           | Total    | 0                           | 12**      | 43** | 49** | 0       | 22** | 49** | 50** |    |
| Pigmentation hepatocytes, centrilobular   | Minimal  | 0                           | 0         | 1    | 26   | 3       | 34   | 34   | 21   |    |
|                                           | Slight   | 0                           | 0         | 0    | 3    | 0       | 0    | 14   | 16   |    |
|                                           | Moderate | 0                           | 0         | 0    | 3    | 0       | 0    | 1    | 8    |    |
|                                           | Marked   | 0                           | 0         | 0    | 0    | 0       | 0    | 0    | 1    |    |
|                                           | Total    | 0                           | 0         | 1    | 32** | 3       | 34** | 49** | 46** |    |
| Eosinophilic hepatocytes, focal           | Minimal  | 6                           | 10        | 18   | 20   | 9       | 14   | 21   | 22   |    |
|                                           | Slight   | 1                           | 0         | 5    | 9    | 0       | 1    | 5    | 7    |    |
|                                           | Moderate | 0                           | 0         | 0    | 3    | 0       | 0    | 0    | 0    |    |
|                                           | Total    | 7                           | 10        | 23** | 32** | 9       | 15   | 26** | 29** |    |
| Hepatocellular vacuolation, centrilobular | Minimal  | 3                           | 7         | 32   | 34   | 1       | 0    | 16   | 3    |    |
|                                           | Slight   | 0                           | 0         | 0    | 4    | 0       | 0    | 1    | 0    |    |
|                                           | Moderate | 0                           | 0         | 0    | 1    | 0       | 0    | 0    | 0    |    |
|                                           | Marked   | 0                           | 0         | 0    | 0    | 0       | 0    | 1    | 0    |    |
|                                           | Total    | 3                           | 7         | 32** | 39** | 0       | 0    | 18** | 3    |    |
| Bile duct hyperplasia <sup>a</sup>        | Minimal  | 3                           | 10        | 18   | 12   | 15      | 11   | 16   | 20   |    |
|                                           | Slight   | 0                           | 1         | 1    | 0    | 0       | 1    | 1    | 1    |    |
|                                           | Moderate | 0                           | 0         | 0    | 0    | 0       | 0    | 0    | 2    |    |
|                                           | Total    | 3                           | 11*       | 19** | 12*  | 15      | 12   | 17   | 23   |    |

**Table 15 (continued)**

|                                                   |         | Severity | Incidence                   |              |              |       |         |        |         |  |
|---------------------------------------------------|---------|----------|-----------------------------|--------------|--------------|-------|---------|--------|---------|--|
|                                                   |         |          | Males                       |              |              |       | Females |        |         |  |
|                                                   |         |          |                             |              |              |       |         |        |         |  |
|                                                   |         |          | Dietary concentration (ppm) |              |              |       |         |        |         |  |
|                                                   |         | 0        | 100                         | 500          | 3000         | 0     | 100     | 500    | 3000    |  |
| Bile duct fibrosis<br><br>(including hyperplasia) | Minimal | 1        | 5                           | 9            | 12           | 6     | 6       | 11     | 11      |  |
|                                                   | Slight  | 0        | 0                           | 1            | 0            | 0     | 0       | 1      | 0       |  |
|                                                   | Total   | 1 (1)    | 5 (5)                       | 10<br>(10)** | 12<br>(10)** | 6 (5) | 6 (5)   | 12 (9) | 11 (11) |  |
| Epithelial mineralization of<br>renal pelvis      | Minimal | 16       | 13                          | 14           | 4            | 38    | 41      | 31     | 9       |  |
|                                                   | Slight  | 2        | 0                           | 0            | 1            | 0     | 0       | 1      | 0       |  |
|                                                   | Total   | 18       | 13                          | 14           | 5**          | 38    | 41      | 32     | 9**     |  |
| Brown pigmentation in<br>kidney tubules           | Minimal | 0        | 1                           | 0            | 0            | 18    | 24      | 30     | 39      |  |
|                                                   | Slight  | 0        | 0                           | 0            | 0            | 0     | 0       | 0      | 3       |  |
|                                                   | Total   | 0        | 1                           | 0            | 0            | 18    | 24      | 30     | 42**    |  |

From Milburn (2008)

\*  $P < 0.05$ ; \*\*  $P < 0.01$ <sup>a</sup> Historical control data from 32 studies: males 25%  $\pm$  17%, range 0–56%; females 31%  $\pm$  18%, range 0–68%.**Table 16. Incidences of neoplastic changes in male and female rats at terminal kill and preterm deaths combined**

|                                    | Incidence                   |           |           |           |           |           |           |           |
|------------------------------------|-----------------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
|                                    | Males                       |           |           |           | Females   |           |           |           |
|                                    | Dietary concentration (ppm) |           |           |           |           |           |           |           |
|                                    | 0                           | 100       | 500       | 3000      | 0         | 100       | 500       | 3000      |
| <i>No. of animals investigated</i> | <i>64</i>                   | <i>64</i> | <i>64</i> | <i>64</i> | <i>64</i> | <i>64</i> | <i>64</i> | <i>64</i> |
| Hepatocellular adenoma             | 1                           | 0         | 0         | 3         | 0         | 1         | 1         | 11**      |
| Hepatocellular carcinoma           | 0                           | 0         | 0         | 1         | 0         | 0         | 0         | 1         |
| Thyroid follicular cell adenoma    | 1                           | 4         | 2         | 7         | 5         | 1         | 3         | 5         |
| Thyroid follicular cell carcinoma  | 0                           | 0         | 5         | 0         | 0         | 1         | 0         | 0         |
| Uterine endometrial adenoma        | —                           | —         | —         | —         | 1         | 0         | 1         | 0         |
| Uterine endometrial adenocarcinoma | —                           | —         | —         | —         | 1         | 2         | 3         | 15**      |

From Milburn (2008)

\*\*  $P < 0.01$ 

The NOAEL was 100 ppm (equal to 5.5 mg/kg bw per day), based on reduced body weight gain in females and increased incidences of foci of eosinophilic hepatocytes and clinical chemistry changes in both sexes at 500 ppm (equal to 27.6 mg/kg bw per day) (Milburn, 2008).

## 2.4 Genotoxicity

Isoprazam was tested for genotoxicity in a range of guideline-compliant assays in vitro and in vivo. No evidence for genotoxicity was observed in any test. The results of the genotoxicity tests are summarized in Table 17. It is concluded that isoprazam is unlikely to be genotoxic.

**Table 17. Results of genotoxicity studies with isopyrazam**

| End-point                        | Test system                                               | Concentration           | Lot No.; purity (%) | Result   | Reference            |
|----------------------------------|-----------------------------------------------------------|-------------------------|---------------------|----------|----------------------|
| Reverse mutation (Ames)          | <i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537 | 5.0–5000 µg/plate (±S9) | SMU6AP001; 96.4     | Negative | Callander (2006)     |
|                                  | <i>Escherichia coli</i> WP2 and WP2 uvrA                  |                         |                     |          |                      |
|                                  | <i>S. typhimurium</i> TA98, TA100, TA1535, TA1537         | 3.0–5000 µg/plate (±S9) | SMU7DP017; 90.8     | Negative | Sokolowski (2008c)   |
|                                  | <i>E. coli</i> WP2 and WP2 uvrA                           |                         |                     |          |                      |
| Mouse lymphoma TK                | L5178Y cell line                                          | 0.63–30 µg/ml (±S9)     | SMU6AP001; 96.4     | Negative | Clay (2006)          |
| Mouse lymphoma TK                | L5178Y cell line                                          | 1.4–66 µg/ml (±S9)      | SMU7DP017; 90.8     | Negative | Wollny (2008c)       |
| Chromosomal aberration           | Human lymphocytes                                         | 10–50 µg/ml (±S9)       | SMU6AP001; 96.4     | Negative | Fox (2006a)          |
| Chromosomal aberration           | Human lymphocytes                                         | 3.0–90.5 µg/ml (±S9)    | SMU7DP017; 90.8     | Negative | Bohnenberger (2008c) |
| Micronucleated bone marrow cells | Male rat                                                  | 2000 mg/kg bw           | SMU6AP001; 96.4     | Negative | Fox (2006c)          |
| Unscheduled DNA synthesis        | Primary hepatocytes of male rats                          | 2000 mg/kg bw           | SMU6AP001; 96.4     | Negative | Fox (2006b)          |

DNA, deoxyribonucleic acid; S9, 9000 × g rat liver supernatant; TK, thymidine kinase

## 2.5 Reproductive toxicity

### (a) Multigeneration studies

#### Rats

Isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 92.8:7.2 *syn:anti*) was administered in the diet to groups of 26 male and 26 female 6-week-old HsdRCCHan:WIST rats. The concentrations in feed were 0, 100, 500 and 3000 ppm. The doses achieved are depicted in Table 18. After a 10-week pre-mating phase under exposure, F<sub>0</sub> animals were mated and allowed to rear two F<sub>1</sub> litters. F<sub>1A</sub> parents were mated after a 10-week pre-mating period and produced a single F<sub>2</sub> generation. Clinical observations, body weight development and feed consumption were recorded regularly (weekly or twice weekly). Reproductive performance was assessed based on successful mating (litter with at least one pup alive), length of gestation and pre-coital interval. F<sub>1A</sub> pups were separated from the dams at postnatal day (PND) 29 and reared as F<sub>1</sub> parents to produce the F<sub>2</sub> generation. Pups were investigated for clinical conditions, survival, body weight development, anogenital distance, vaginal opening and preputial separation. At termination of F<sub>0</sub> and F<sub>1</sub> parents, organ weights and histology, sperm number, morphology, motility and spermatid resistance to homogenization were recorded. In pups, organs were weighed and examined macroscopically.

There were no significant clinical observations in F<sub>0</sub> or F<sub>1</sub> parental animals. Body weights in F<sub>0</sub> and F<sub>1</sub> animals were lower in females at 500 and 3000 ppm and in males at 3000 ppm throughout pre-mating and gestation. After parturition, body weights in the female 500 ppm group were similar to those of controls.

The number of estrous cycles in the 21-day observation period was statistically significantly higher in F<sub>1</sub> females in the 100 and 500 ppm groups than in the control group, but in the absence of a similar finding in the high-dose group and as the number of cycles in all groups was generally four or five, this is considered to be an incidental finding.

**Table 18. Doses achieved in the multigeneration study in rats**

|                                                | Doses (mg/kg bw per day) |         |          |         |         |          |
|------------------------------------------------|--------------------------|---------|----------|---------|---------|----------|
|                                                | Males                    |         |          | Females |         |          |
|                                                | 100 ppm                  | 500 ppm | 3000 ppm | 100 ppm | 500 ppm | 3000 ppm |
| F <sub>0</sub> parents pre-mating              | 8.3                      | 41.2    | 250.1    | 9.3     | 46.6    | 276.6    |
| F <sub>1</sub> parents pre-mating              | 9.5                      | 47.8    | 288.5    | 10.2    | 50.1    | 301.3    |
| F <sub>0</sub> females during gestation        | —                        | —       | —        | 7.4     | 37.2    | 217.4    |
| F <sub>1</sub> females during gestation        | —                        | —       | —        | 8.1     | 40.6    | 239.1    |
| F <sub>0</sub> females postpartum <sup>a</sup> | —                        | —       | —        | 25.2    | 118.9   | 699.6    |
| F <sub>1</sub> females postpartum <sup>a</sup> | —                        | —       | —        | 24.4    | 129.1   | 774.0    |

From Milburn (2007d)

<sup>a</sup> Feed consumption postpartum assumes that the dam consumed all the feed, although the pups present in the cage are known to start to consume diet from around week 2.

F<sub>1A</sub> and F<sub>2A</sub> pup body weights were reduced by maximally 14–21% from PND 15 onwards at 3000 ppm, and mean total litter weights were reduced by 21–23% (averaged over PNDs 1–29). At 500 ppm, mean total litter weights were reduced by 7–8% (averaged over PNDs 1–29). In the 100 ppm group, no effect was observed. F<sub>1</sub> males at 3000 ppm showed statistically significantly delayed preputial (2.3 days) separation, and F<sub>1</sub> females at 3000 ppm showed statistically significantly delayed vaginal opening (2 days). Whereas the males showed statistically significantly reduced body weights (–7%), the body weights of females were unchanged.

Liver weights were increased statistically significantly at 3000 ppm in F<sub>0</sub> males and at 500 ppm and above in F<sub>1</sub> males. In females, liver weights were statistically significantly higher at 500 ppm and above in F<sub>0</sub> animals and at 100 ppm and above in F<sub>1</sub> animals. In F<sub>1</sub> and F<sub>2</sub> pups of both sexes, liver weights were statistically significantly increased at 500 ppm and above. Minimal or slight centrilobular or diffuse hepatocellular hypertrophy was dose-relatedly increased in F<sub>0</sub> and F<sub>1</sub> animals at 500 and 3000 ppm. In F<sub>0</sub> males at 3000 ppm, seminal vesicle weights were statistically significantly lower by 10% compared with controls. In F<sub>0</sub> males, thyroid weights were increased statistically significantly at 500 ppm by 14% and at 3000 ppm by 43%. In F<sub>0</sub> and F<sub>1</sub> 3000 ppm females, weights of uterus with cervix were decreased statistically significantly by 27–41%. In F<sub>1</sub> females at 500 and 3000 ppm, adrenal weights were higher by 12–14%. Kidney weights in F<sub>1</sub> animals were dose-relatedly increased at all dose levels in both sexes, statistically significantly in females at all dose levels and in males at 3000 ppm. Ovary weights in high-dose F<sub>0</sub> and F<sub>1</sub> females were statistically significantly lower by 13–27%.

The number of implantations and the number of live and dead fetuses combined were both statistically significantly lower in F<sub>0</sub> and F<sub>1</sub> dams at 3000 ppm compared with the controls.

The NOAELs for parental and postnatal developmental toxicity were both 100 ppm (equal to 8.1 mg/kg bw per day), based on decreased body weight and organ weight changes in parental F<sub>0</sub> and F<sub>1</sub> animals (parental toxicity) and on lower mean total litter weights (postnatal developmental toxicity) at 500 ppm (equal to 40.6 mg/kg bw per day). The NOAEL for reproductive performance was 3000 ppm (equal to 239.1 mg/kg bw per day), the highest dose tested (Milburn, 2007d).

#### (b) Developmental toxicity

##### Rats

Isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 92.8:7.2 *syn:anti*) was administered by oral gavage from gestation day (GD) 5 to GD 21 to groups of 24 female time-mated

11- to 13-week-old HsdRCCHan:WIST rats. The dose levels were 0, 20, 75 and 250 mg/kg bw per day. Detailed clinical observations, body weights and feed consumption were recorded. All rats terminated at day 22 of gestation were given a macroscopic examination, including external observation and an examination of the thoracic and abdominal viscera. The following developmental parameters were examined: number of corpora lutea, gravid uterus weight, number and position of implantations, number of live fetuses, number of early and late intrauterine deaths, weight and sex of fetuses and external, visceral and skeletal abnormalities and variations.

Two animals in the 250 mg/kg bw per day group were killed on days 20 and 21, respectively, as they had marked body weight loss and severe clinical signs of toxicity. One of these animals may have been dosed into the thorax. In the high-dose group, the body weights and feed consumption were statistically significantly lower than in the control groups throughout the study. Pre-implantation losses, post-implantation losses, early intrauterine deaths and consequently the mean number of live fetuses per litter, mean gravid uterus weights and litter weights were affected in a dose-related manner, statistically significantly compared with the concurrent control group (Table 19). However, the historical control data of five developmental toxicity studies in the same year show that the concurrent control values for pre-implantation losses, post-implantation losses and early intrauterine deaths were lower than those for any of the other control groups and that the treated groups showed values similar to the historical control values. Therefore, these observations are regarded as incidental. At 250 mg/kg bw per day, the mean fetal body weight was statistically significantly decreased. At all dose levels, male fetuses were under-represented compared with the concurrent and the historical control groups.

In the 250 mg/kg bw per day group, one fetus with hydroencephalus and microphthalmia was observed, and in another litter, a fetus with internal hydrocephalus only was noted (historical controls for microphthalmia: 0/1354 fetuses; historical controls for internal hydrocephali: 0/1354 fetuses). Whereas incompletely ossified structures in the skull were observed at statistically significantly lower incidences at 75 mg/kg bw per day and above than in controls, non-ossified cervical centra and incomplete xiphoid cartilage were observed at statistically significantly higher incidences. At 250 mg/kg bw per day, incidences of incompletely or non-ossified caudal arches and hind paws were increased statistically significantly.

The NOAEL for maternal toxicity was 75 mg/kg bw per day, based on reduced body weight and clinical signs of toxicity at 250 mg/kg bw per day. The NOAEL for developmental toxicity was 20 mg/kg bw per day, based on non-ossified cervical centra at 75 mg/kg bw per day (Moxon, 2007).

Isoprazam (SYN520453, batch No. SMU7DP017, purity 90.8%, 69.7:30.3 *syn:anti*) was administered by oral gavage from GD 4 to GD 20 to groups of 24 female time-mated 11-week-old RCCHan:WIST rats. The dose levels were 0, 20, 75 and 200 mg/kg bw per day. Detailed clinical observations, body weights and feed consumption were recorded. All rats terminated at day 21 of gestation were examined externally, and organs were investigated macroscopically. The following developmental parameters were examined: number of corpora lutea, gravid uterus weight, number and position of implantations, number of live fetuses, number of early and late intrauterine deaths, weight and sex of fetuses, and external, visceral and skeletal abnormalities and variations.

At 200 mg/kg bw per day, ventral recumbency and sedation were noted in all 24 dams from the 1st day of treatment throughout the 1st week. Thereafter, these effects and ruffled fur were seen occasionally in a few animals. Feed consumption was reduced statistically significantly by 15% from the 1st day of treatment (GD 4) in animals at 75 mg/kg bw per day and above; concomitantly, the body weight gain was reduced statistically significantly by 5% from the 4th day of treatment in the 75 mg/kg bw per day group. At 200 mg/kg bw per day, animals lost weight from the 2nd day of treatment up to GD 10 and then gained body weight to a modest extent. The final body weight in this group was lower by 5% at 75 mg/kg bw per day and by 17% at 200 mg/kg bw per day.

**Table 19. Litter data in a developmental toxicity study in rats**

|                                                                   | Dose (mg/kg bw per day) |        |       |        | Historical control data <sup>a</sup> |
|-------------------------------------------------------------------|-------------------------|--------|-------|--------|--------------------------------------|
|                                                                   | 0                       | 20     | 75    | 250    |                                      |
| No. of animals investigated                                       | 23                      | 24     | 23    | 22     |                                      |
| Mean live fetuses per litter                                      | 13.1                    | 12.0   | 11.6* | 11.3** |                                      |
| Mean gravid uterus weight (g)                                     | 84.3                    | 78.8   | 76.7* | 68.5** |                                      |
| Litter weight (g)                                                 | 62.0                    | 57.8   | 55.6* | 50.0** |                                      |
| Mean fetal weight (g)                                             | 4.76                    | 4.86   | 4.84  | 4.42*  |                                      |
| Proportion of male fetuses                                        | 54.9                    | 43.8*  | 44.8  | 44.5*  | 47.1, 49.8, 48.0, 50.5, 50.8         |
| Pre-implantation losses, implants affected (%)                    | 1.9                     | 8.6**  | 7.7*  | 8.1**  | 7.6, 7.3, 13.0, 6.7, 2.7             |
| Pre-implantation losses, litters affected (%)                     | 21.7                    | 62.5** | 52.2  | 59.0*  |                                      |
| Post-implantation losses, implants affected (%)                   | 1.6                     | 4.2    | 3.8   | 8.4**  | 5.8, 3.2, 9.7, 5.0, 4.0              |
| Post-implantation losses, litters affected (%)                    | 21.7                    | 33.3   | 30.4  | 59.1*  |                                      |
| Pre- and post-implantation losses combined, implants affected (%) | 3.5                     | 12.2   | 11.3  | 16.7   |                                      |
| Early intrauterine deaths, implants affected (%)                  | 1                       | 4.2    | 3.8   | 7.5**  | 5.0, 1.1, 7.8, 4.0, 4.0              |
| Early intrauterine deaths, litters affected (%)                   | 13                      | 33.3   | 30.4  | 54.5   |                                      |
| Late intrauterine deaths, implants affected (%)                   | 0.6                     | 0      | 0     | 0.9    | 0.7, 2.1, 1.9, 1.1, 0                |
| Late intrauterine deaths, litters affected (%)                    | 8.7                     | 0      | 0     | 4.5    |                                      |

From Moxon (2007)

\*  $P < 0.05$ ; \*\*  $P < 0.01$ <sup>a</sup> Means of five developmental toxicity studies in 2006.

No effects on implantation rates, pre-implantation and post-implantation losses or the number of live fetuses were found. At 75 mg/kg bw per day and above, fetal body weights were statistically significantly lower compared with controls. One fetus at 200 mg/kg bw per day was found with diaphragmatic hernia (historical controls: 1/4277 fetuses). Statistically significantly increased incidences of delayed or absent ossification of cervical vertebrae bodies were observed at 200 mg/kg bw per day and of incompletely ossified sternebrae at 75 mg/kg bw per day and above. Additionally, non-ossified structures in forelimbs and hindlimbs were identified.

The NOAELs for maternal and developmental toxicity were both 20 mg/kg bw per day, based on clinical signs and reduced body weight gain in dams (maternal toxicity) and lower fetal body weights (developmental toxicity) at 75 mg/kg bw per day (Possnecker, 2008).

### *Rabbits*

In a dose range-finding developmental toxicity study, isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 92.8:7.2 *syn:anti*) was administered by oral gavage from GD 4 to GD 27 to groups of 10 female (> 17 weeks old) mated Himalayan rabbits. The dose levels were 0, 100, 200 and 400 mg/kg bw per day. Clinical observations, body weights and feed consumption were recorded. All rabbits were terminated at GD 28, and gross macroscopic examination of all internal organs, with emphasis on the uterus, uterine contents, position of fetuses in the uterus and number of corpora lutea, was performed. The uteri (and contents) with live fetuses were weighed. Fetuses were weighed individually and examined for gross external and visceral abnormalities, and the cranium was examined for the degree of ossification. No histopathological examinations of the heads were performed.

There were no effects on feed consumption, body weight gain, implantation rates, pre-implantation and post-implantation losses or number of live fetuses. Sex ratios and fetal body weights

were not affected. In fetuses at 400 mg/kg bw per day, a range of cardiovascular changes, including interventricular septal defects, were observed in 7 of 57 fetuses (6/9 litters affected). In controls, no fetuses were affected, and at the low and middle dose levels, two fetuses and one fetus were affected, respectively. Two high-dose fetuses had slightly smaller eyes compared with controls. Two high-dose fetuses and one mid-dose fetus had multiple vertebral, sternebrae and/or rib abnormalities or slightly asymmetrical skull with frontal to parietal bones fused. On a litter basis, statistically significant increases in the incidence of a branched xiphoid cartilage in the cartilaginous sternum were noted at 200 and 400 mg/kg bw per day. However, on a fetal basis, the incidence was not increased.

In this dose range-finding study, the NOAEL for maternal toxicity was 400 mg/kg bw per day, the highest dose tested. The NOAEL for fetal toxicity was 200 mg/kg bw per day, based on the incidence of small eyes and other abnormalities at 400 mg/kg bw per day (Gerspach, 2008a).

In a second dose range-finding developmental toxicity study, isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 92.8:7.2 *syn:anti*) was administered by oral gavage from GD 4 to GD 27 to groups of five female (> 17 weeks old) mated Himalayan rabbits. The dose levels were 0, 600, 800 and 1000 mg/kg bw per day. Clinical observations, body weights and feed consumption were recorded. All rabbits were terminated at GD 28, and gross macroscopic examination of all internal organs, with emphasis on the uterus, uterine contents, position of fetuses in the uterus and number of corpora lutea, was performed. The uteri (and contents) with live fetuses were weighed. Fetuses were weighed individually and examined for gross external and visceral abnormalities, and the cranium was examined for the degree of ossification. Additionally, histopathological examinations of the heads were performed.

Animals at 1000 mg/kg bw per day had slightly lower feed consumption, but body weight gain was comparable to that of controls. Parental necropsy and reproductive parameters were not affected. The sex ratio of fetuses was unchanged. At 1000 mg/kg bw per day, male fetuses had slightly, statistically not significantly lower body weights. At all dose levels, eyes smaller than usually observed were found, and a higher incidence of fetuses with malrotated or flexed limbs was observed (Table 20). At 800 mg/kg bw per day and above, changes in the skull were found, and at 1000 mg/kg bw per day, two fetuses with abnormal lung lobulation and one fetus each with an additional liver lobe and absent kidney and ureter were observed.

The NOAEL for maternal toxicity was 1000 mg/kg bw per day, the highest dose tested. Fetotoxicity as small eyes and malrotated or flexed limbs was observed at all dose levels (Gerspach, 2008b).

In a third dose range-finding developmental toxicity study, isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 92.8:7.2 *syn:anti*) was administered by oral gavage from GD 7 to GD 28 to groups of 10 female (24–26 weeks old) mated New Zealand White rabbits. The dose levels were 0, 400, 700 and 1000 mg/kg bw per day. Clinical observations, body weights and feed consumption were recorded. All rabbits were terminated at GD 29, blood samples were taken for clinical chemistry and gross macroscopic examination of all internal organs (including microscopy of livers), with emphasis on the uterus, uterine contents, positions of fetuses in the uterus and the number of corpora lutea, was performed. The uteri (and contents) with live fetuses were weighed. Fetuses were weighed individually and examined for gross external, visceral and skeletal abnormalities.

All treated groups had statistically significantly lower body weights (–4%) compared with controls and dose-relatedly lower feed consumption (by 13–33%). In the mid- and high-dose groups, one dam was killed in extremis at each of GD 23 and GD 21, respectively, after having lost appreciable body weight. One low-dose and one mid-dose dam aborted on GD 25. One high-dose dam had significantly elevated GGT activity, and cholangiofibrosis was noted in this dam at termination. In high-

**Table 20. Fetal data in the second dose range-finding developmental toxicity study in rabbits**

|                                        | Incidence of finding    |   |      |   |     |   |      |     | Historical control data <sup>a</sup> |
|----------------------------------------|-------------------------|---|------|---|-----|---|------|-----|--------------------------------------|
|                                        | Dose (mg/kg bw per day) |   |      |   |     |   |      |     |                                      |
|                                        | 0                       |   | 600  |   | 800 |   | 1000 |     |                                      |
|                                        | F                       | L | F    | L | F   | L | F    | L   |                                      |
| <i>No. of fetuses/litters examined</i> | 33                      | 5 | 27   | 5 | 23  | 5 | 32   | 5   |                                      |
| Skull                                  |                         |   |      |   |     |   |      |     |                                      |
| - frontoparietal suture irregular      | 0                       | 0 | 0    | 0 | 1   | 1 | 2    | 2   |                                      |
| - bones fused                          | 0                       | 0 | 0    | 0 | 1   | 1 | 1    | 1   | 8 (1.2%)                             |
| - irregular ossification               | 0                       | 0 | 0    | 0 | 0   | 0 | 2    | 2   | 7 (1%)                               |
| Limbs flexed or malrotated             | 0                       | 0 | 1    | 1 | 2   | 1 | 4    | 4** | 22 (3.2%)                            |
| Lung abnormal lobulation               | 0                       | 0 | 0    | 0 | 0   | 0 | 2    | 1   |                                      |
| Liver additional lobe                  | 0                       | 0 | 0    | 0 | 0   | 0 | 1    | 1   | 4 (0.6%)                             |
| Kidney and ureter absent               | 0                       | 0 | 0    | 0 | 0   | 0 | 1    | 1   | 3 (0.4%)                             |
| Eye small                              |                         |   |      |   |     |   |      |     |                                      |
| - 50–75% of normal size                | 0                       | 0 | 2    | 1 | 0   | 0 | 5**  | 3   |                                      |
| - 75–< 100% of normal size             | 0                       | 0 | 9**  | 3 | 5** | 2 | 10** | 3   |                                      |
| - total small eyes                     | 0                       | 0 | 10** | 3 | 5** | 2 | 14** | 3   | 1 (0.15%)                            |

From Gerspach (2008b)

F, fetus; L, litter; \*\*  $P < 0.01$ <sup>a</sup> Historical control data from five control groups with 682 fetuses in 101 litters.

dose animals, slightly elevated urea nitrogen levels were noted. The relative liver weights of animals in all dosed groups were increased by 33–66%. Histologically, mid- and high-dose animals had hepatocellular hypertrophy, and the cytoplasm of hepatocytes contained vacuoles, most probably lipid containing. At 1000 mg/kg bw per day, early resorptions were statistically significantly increased, and mean fetal weights were lower than control weights. Small eyes were noted in 1 of 95 control fetuses and 5 of 76 fetuses at 1000 mg/kg bw per day (Table 21). Several ocular changes were observed in three of the five high-dose fetuses with small eyes; the changes included haemorrhagic rings around the iris, reddened eyes and dark areas on the eyes. Furthermore, absent gall bladders, extra papillary muscle in the heart and variations of major blood vessels were observed at all dose levels.

No maternal or developmental NOAEL was identified in this study, as reduced body weight in dams and possibly treatment-related absence of gall bladder in fetuses were observed at all dose levels (Sawhney Coder, 2008b).

In a developmental toxicity study, isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 92.8:7.2 *syn:anti*) was administered by oral gavage from GD 7 to GD 28 to groups of 25 female 25-week-old mated New Zealand White rabbits. The dose levels were 0, 30, 150 and 500 mg/kg bw per day. Clinical observations, body weights and feed consumption were recorded. All rabbits were terminated at GD 29, and gross macroscopic examination of all internal organs (including histology of livers), with emphasis on the uterus, uterine contents, positions of fetuses in the uterus and the number of corpora lutea, was performed. The uteri (and contents) with live fetuses were weighed. Fetuses were weighed individually and examined for external, visceral and skeletal abnormalities.

One high-dose dam died on GD 24 after having shown signs of intoxication throughout the study. This female had red discoloured lungs and nine late resorptions. Maternal body weight gain was unaffected in all dose groups. At GDs 7–17, feed consumption in the 500 mg/kg bw per day

**Table 21. Fetal data in the third dose range-finding developmental toxicity study in rabbits**

|                                        | Dose (mg/kg bw per day) |    |      |   |     |   |      |   |
|----------------------------------------|-------------------------|----|------|---|-----|---|------|---|
|                                        | 0                       |    | 400  |   | 700 |   | 1000 |   |
|                                        | F                       | L  | F    | L | F   | L | F    | L |
| <i>No. of fetuses/litters examined</i> | 95                      | 10 | 62   | 8 | 66  | 8 | 76   | 9 |
| Major blood vessel variation           | 1                       | 1  | 3    | 2 | 2   | 1 | 6    | 3 |
| Extra papillary muscle in heart        | 6                       | 4  | 15** | 4 | 7   | 5 | 13** | 8 |
| Gall bladder absent <sup>a</sup>       | 1                       | 1  | 5*   | 3 | 6*  | 3 | 11** | 5 |
| Eye small                              | 1                       | 1  | 0    | 0 | 0   | 0 | 5    | 2 |

From Sawhney Coder (2008b)

F, fetus; L, litter; \*  $P < 0.05$ ; \*\*  $P < 0.01$

<sup>a</sup> Historical control data: mean 3.9%; median 3.0%; 75th percentile 5.1%, range 0–12%.

group was slightly lower than in the control group, and this was associated with reduced defecation. Absolute liver weights were increased in the 150 mg/kg bw per day group by 13.1% and in the 500 mg/kg bw per day group by 36.2%. At 150 and 500 mg/kg bw per day, dose-related increases in the incidence and severity of hepatocellular hypertrophy and cytoplasmic vacuolation were noted (Table 22). The vacuoles were free of polyribosomes and probably represented lipid accumulation.

Slightly lower fetal body weights in the 500 mg/kg bw per day group were probably secondary to the slightly higher litter size. One fetus in the 500 mg/kg bw per day group had bilateral microphthalmia. There were no other dose-related changes in the incidences of external, visceral or skeletal malformations or variations.

The NOAEL for maternal toxicity was 150 mg/kg bw per day, based on a statistically significantly increased incidence of centrilobular hepatocellular vacuolation at 500 mg/kg bw per day. The developmental NOAEL was 150 mg/kg bw per day, based on a single observation of bilateral microphthalmia at 500 mg/kg bw per day. It was noted that although the incidence of this defect was within the historical control range, a treatment-related increase in the incidence of this defect had been observed at higher doses in the range-finding studies (Sawhney Coder, 2008c).

## 2.6 Special studies

### (a) Neurotoxicity

#### Rats

In an acute neurotoxicity study, a single dose of isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 92.8:7.2 *syn:anti*) was administered by gavage to groups of 10 male and 10 female 7-week-old HanRCC:WIST rats. The dose levels were 0, 30, 250 and 2000 mg/kg bw. Animals were observed for 15 days. Detailed clinical observations, body weights and feed consumption were recorded weekly. A FOB was performed, and locomotor activity was recorded. At day 16, animals were killed, and the brains were weighed and preserved together with spinal cord and samples from the peripheral nervous system for microscopic examination.

At 1 hour post-dosing, animals had weak appearance and decreased activity in the 250 and 2000 mg/kg bw groups. In these groups, locomotor activity was also reduced 3 hours post-dosing on the 1st day. These effects were dose dependent in incidence and severity. Body weight gain was reduced during the 1st week after treatment in the 250 and 2000 mg/kg bw groups.

**Table 22. Liver histology in rabbit dams in a developmental toxicity study**

| Histological finding                     | Severity | Incidence of finding    |              |     |      |
|------------------------------------------|----------|-------------------------|--------------|-----|------|
|                                          |          | Dose (mg/kg bw per day) |              |     |      |
|                                          |          | 0                       | 30           | 150 | 500  |
| <i>No. of animals investigated</i>       |          | 23                      | 24           | 23  | 23   |
| Hepatocellular hypertrophy               | Minimal  | 0                       | <sup>a</sup> | 4   | 12   |
|                                          | Mild     | 0                       | <sup>a</sup> | 0   | 2    |
|                                          | Total    | 0                       | <sup>a</sup> | 4*  | 14** |
| Centrilobular hepatocellular vacuolation | Minimal  | 0                       | <sup>b</sup> | 3   | 8    |
|                                          | Mild     | 0                       | <sup>b</sup> | 0   | 2    |
|                                          | Moderate | 0                       | <sup>b</sup> | 0   | 3    |
|                                          | Total    | 0                       | <sup>b</sup> | 3   | 13** |
| Midzonal hepatocellular vacuolation      | Minimal  | 4                       | <sup>b</sup> | 2   | 5    |
|                                          | Mild     | 2                       | <sup>b</sup> | 3   | 0    |
|                                          | Moderate | 0                       | <sup>b</sup> | 0   | 1    |
|                                          | Total    | 6                       | <sup>b</sup> | 5   | 6    |

From Sawhney Coder (2008c)

\*  $P < 0.05$ ; \*\*  $P < 0.01$

<sup>a</sup> Histological evaluation indicated that hepatocyte hypertrophy alteration in four rabbits in the 30 mg/kg bw per day group was minor and less than the minimal grade in the 150 and 500 mg/kg bw per day groups.

<sup>b</sup> No data given.

The NOAEL for systemic effects was 30 mg/kg bw, based on clinical signs of toxicity at 250 mg/kg bw. The NOAEL for acute neurotoxicity was 2000 mg/kg bw, the highest dose tested (Sommer, 2009b).

In a subchronic neurotoxicity study, isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 92.8:7.2 *syn:anti*) was administered for 90 days in the diet to groups of 12 male and 12 female 7-week-old HanRCC:WIST rats. The concentrations in feed were 0, 300, 1500 and 6000 ppm. The eyes of all animals were examined pre-study, and the eyes of the control and high-dose groups were examined during the week prior to termination. Detailed clinical observations and body weights were recorded weekly, and feed consumption was recorded twice weekly. A FOB was performed, and locomotor activity was recorded. At week 13, animals were killed, and the brains were weighed and preserved together with spinal cord and samples from the peripheral nervous system for microscopic examination.

Feed consumption was reduced in male and female rats at 6000 ppm, in males by 4% in the 1st week only and in females by 12% during the first half of the study. Concomitantly, body weight gain was reduced in males during the first weeks and in females by 10% throughout the whole study. No other treatment-related effects were observed.

The NOAEL for systemic toxicity was 1500 ppm (equal to 98.01 mg/kg bw per day), based on decreased body weight gain in females at 6000 ppm (equal to 382.26 mg/kg bw per day). The NOAEL for subchronic neurotoxicity was 6000 ppm (equal to 382.26 mg/kg bw per day), the highest dose tested (Sommer, 2009a).

*(b) Induction of enzyme activity, DNA synthesis and estrogen receptor alpha binding*

Isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 93:7 *syn:anti*) was tested for its ability to induce CYP2B (PROD) and CYP2B/3A (benzyloxyresorufin *O*-debenzylase [BROD])

activity and cell proliferation (measured as the change in DNA synthesis [S-phase of the cell cycle]) in isolated primary hepatocytes of female Han Wistar rats. Phenobarbital sodium salt was used as positive control. Hepatocytes were exposed to isopyrazam at 1, 3, 10, 30, 65 and 100  $\mu\text{mol/l}$  and phenobarbital sodium salt at 10, 100 and 1000  $\mu\text{mol/l}$  or vehicle (0.5% DMSO) for 96 hours. Cytotoxicity was assayed by measuring the change in cellular adenosine-5'-triphosphate (ATP) levels.

Both phenobarbital sodium salt and isopyrazam induced PROD and BROD activities and DNA synthesis. Comparing the potencies using the per cent changes at the lowest doses producing a statistically significant effect gives a higher potency of isopyrazam compared with phenobarbital sodium salt, for PROD 17-fold, for BROD 22-fold and for DNA synthesis 12-fold (Table 23) (Elcombe, 2011d).

Isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 93:7 *syn:anti*) was tested for its ability to induce CYP2B (PROD) and CYP2B/3A (BROD) activities and cell proliferation (measured as the change in DNA synthesis [S-phase of the cell cycle]) in isolated primary human female hepatocytes. Phenobarbital sodium salt was used as positive control. Hepatocytes were exposed to isopyrazam at 1, 3, 10, 30, 65 and 100  $\mu\text{mol/l}$  and phenobarbital sodium salt at 10, 100 and 1000  $\mu\text{mol/l}$  or vehicle (0.5% DMSO) for 96 hours. Cytotoxicity was assayed by measuring the change in cellular ATP levels.

Phenobarbital sodium salt and isopyrazam did not induce DNA synthesis statistically significantly at any concentration level. Phenobarbital sodium salt statistically significantly induced PROD and BROD activities at 1000  $\mu\text{mol/l}$ . Isopyrazam did not statistically significantly induce PROD activity even at the highest concentration but was a potent inducer of BROD activity at all dose levels tested (Table 24). Comparing the potencies using the per cent changes at the lowest doses producing a statistically significant effect gives a 470-fold higher potency of isopyrazam compared with phenobarbital sodium salt for BROD activity (Elcombe, 2011c).

Isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 93:7 *syn:anti*) was tested for its ability to induce activities of CYP1A (EROD), CYP2B (PROxD), CYP2B/3A (BROD), CYP3A (testosterone 6 $\beta$ -hydroxylase) and CYP4A (lauric acid 12-hydroxylase) and cell proliferation (measured as the change in DNA synthesis [S-phase of the cell cycle]) in 5- to 6-week-old female Han Wistar rats. Additionally, detailed clinical observations were recorded weekly, and body weight and feed consumption were recorded daily. ALT and AST activities were measured, and livers were weighed and investigated microscopically. At feed concentrations of 0, 500 or 3000 ppm each, 10 animals were fed for 3, 7 or 14 days.

At several time points, animals in the 500 and 3000 ppm groups showed lower feed intake. In the 3000 ppm group, the body weight gain was initially low, but recovered thereafter. No changes in ALT and AST activities were observed. Relative liver weights were increased from day 4 onwards at 500 and 3000 ppm by approximately 10% and 20%, respectively. At 3000 ppm, 9 of 10 animals showed histologically increased mitosis in the liver at day 4 in the 3000 ppm group, and all animals had minimal to mild hepatocellular hypertrophy at days 8 and 15. The liver S-phase labelling index was elevated 1.5-fold at 500 ppm at day 3 (statistically not significant) and about 2.1-fold at 3000 ppm. At days 8 and 15, S-phase labelling indices were all similar. At both dose levels and at all time points, total CYP content and CYP1A and CYP2B activities were statistically significantly elevated. For CYP1A and CYP2B activities, there was no significant difference in induction potency between the two dose levels. For CYP3A, there was a dose-related decrease in enzyme activity at all three time points (Table 25). There was a slight and statistically significant decrease (17–30%) in peroxisomal palmitoyl coenzyme A metabolism in the 300 ppm group at all time points. The effect was statistically significant at day 3 and day 7 (Murchison, 2010).

**Table 23. Change in enzyme activity and DNA synthesis in cultured primary female rat hepatocytes**

| Treatment                  | Control           | Phenobarbital sodium salt           |                    |                    |                    |                    | Isopyrazam         |                    |                               |  |  |
|----------------------------|-------------------|-------------------------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|-------------------------------|--|--|
|                            |                   | Concentration ( $\mu\text{mol/l}$ ) |                    |                    |                    |                    |                    |                    |                               |  |  |
|                            | 0                 | 10                                  | 100                | 1000               | 1                  | 3                  | 10                 | 30                 | 65                            |  |  |
| ATP <sup>a</sup>           | 100.0 $\pm$ 3.2   | 101.7 $\pm$ 1.7                     | 104.9 $\pm$ 5.0    | 113.1 $\pm$ 3.7**  | 105.6 $\pm$ 3.0*   | 106.6 $\pm$ 4.1*   | 106.4 $\pm$ 3.5**  | 96.7 $\pm$ 4.7     | 2.0 $\pm$ 0.1**               |  |  |
| DNA synthesis <sup>b</sup> | 100.0 $\pm$ 11.1  | 164.9 $\pm$ 19.3**                  | 215.9 $\pm$ 16.1** | 242.7 $\pm$ 7.2**  | 192.2 $\pm$ 11.7** | 257.4 $\pm$ 20.8** | 226.4 $\pm$ 22.4** | 218.4 $\pm$ 43.7** | 194.5 $\pm$ 26.3 <sup>c</sup> |  |  |
| PROD <sup>d</sup>          | 100.0 $\pm$ 153.8 | 109.8 $\pm$ 45.8                    | 395.5 $\pm$ 73.7*  | 584.3 $\pm$ 67.5** | 147.0 $\pm$ 96.9   | 289.6 $\pm$ 97.1   | 664.3 $\pm$ 190.8* | 408.0 $\pm$ 19.8*  | 286.0 $\pm$ 74.7 <sup>c</sup> |  |  |
| BROD <sup>d</sup>          | 100.0 $\pm$ 13.6  | 110.4 $\pm$ 19.4                    | 202.8 $\pm$ 16.2** | 261.6 $\pm$ 49.3** | 135.2 $\pm$ 67.8   | 136.9 $\pm$ 14.0*  | 221.4 $\pm$ 28.6** | 274.2 $\pm$ 56.9** | 111.8 $\pm$ 16.4 <sup>c</sup> |  |  |

From Elcombe (2011d)

ATP, adenosine-5'-triphosphate; BROD, benzyloxyresorufin O-debenzylase; DNA, deoxyribonucleic acid; PROD, 7-pentoxoresorufin O-dealkylase; \*  $P < 0.05$ ; \*\*  $P < 0.01$ <sup>a</sup>  $n = 6$  per group.<sup>b</sup>  $n = 5$  per group.<sup>c</sup> No statistical analyses performed (excessive cytotoxicity).<sup>d</sup>  $n = 3$  per group.**Table 24. Change in enzyme activity compared with control and DNA synthesis in cultured primary female human hepatocytes**

| Treatment                  | Control          | Phenobarbital sodium salt           |                  |                    |                    |                    | Isopyrazam         |                    |  |  |  |
|----------------------------|------------------|-------------------------------------|------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--|--|--|
|                            |                  | Concentration ( $\mu\text{mol/l}$ ) |                  |                    |                    |                    |                    |                    |  |  |  |
|                            | 0                | 10                                  | 100              | 1000               | 1                  | 3                  | 10                 | 30                 |  |  |  |
| ATP <sup>a</sup>           | 100.0 $\pm$ 7.2  | 108.8 $\pm$ 11.5                    | 106.0 $\pm$ 9.2  | 95.5 $\pm$ 10.4    | 101.1 $\pm$ 8.4    | 98.1 $\pm$ 5.8     | 89.1 $\pm$ 5.3*    | 64.6 $\pm$ 7.7**   |  |  |  |
| DNA synthesis <sup>b</sup> | 100.0 $\pm$ 38.3 | 122.7 $\pm$ 10.4                    | 121.6 $\pm$ 40.3 | 161.7 $\pm$ 51.7   | 85.9 $\pm$ 15.2    | 102.9 $\pm$ 37.1   | 110.8 $\pm$ 21.8   | 117.3 $\pm$ 22.9   |  |  |  |
| PROD <sup>c</sup>          | 100.0 $\pm$ 24.2 | 73.8 $\pm$ 10.0                     | 70.5 $\pm$ 20.9  | 183.0 $\pm$ 14.1** | 92.5 $\pm$ 36.6    | 58.8 $\pm$ 30.3    | 85.9 $\pm$ 33.7    | 167.9 $\pm$ 60.5   |  |  |  |
| BROD <sup>c</sup>          | 100.0 $\pm$ 27.7 | 124.3 $\pm$ 12.9                    | 193.5 $\pm$ 87.9 | 632.4 $\pm$ 32.4** | 294.9 $\pm$ 15.5** | 383.9 $\pm$ 94.3** | 420.8 $\pm$ 46.0** | 490.3 $\pm$ 96.0** |  |  |  |

From Elcombe (2011c)

ATP, adenosine-5'-triphosphate; BROD, benzyloxyresorufin O-debenzylase; DNA, deoxyribonucleic acid; PROD, 7-pentoxoresorufin O-dealkylase; \*  $P < 0.05$ ; \*\*  $P < 0.01$ <sup>a</sup>  $n = 6$  per group.<sup>b</sup>  $n = 5$  per group.<sup>c</sup>  $n = 3$  per group.

**Table 25. Change in enzyme activity relative to control in liver microsomes**

|                  | % change relative to control |      |      |         |         |         |              |     |     |       |     |     |
|------------------|------------------------------|------|------|---------|---------|---------|--------------|-----|-----|-------|-----|-----|
|                  | CYP1A                        |      |      | CYP2B   |         |         | CYP3A        |     |     | CYP4A |     |     |
|                  | Day                          |      |      |         |         |         |              |     |     |       |     |     |
|                  | 3                            | 8    | 15   | 3       | 8       | 15      | 3            | 8   | 15  | 3     | 8   | 15  |
| 500 ppm          | 328*                         | 247* | 242* | 30 303* | 11 048* | 20 912* | 109          | 100 | 84  | 101   | 113 | 95  |
| 3000 ppm         | 213*                         | 255* | 269* | 34 640* | 16 195* | 33 953* | <sup>b</sup> | 74  | 59* | 78*   | 104 | 111 |
| 3-MC             | 7750                         | ne   | ne   | ne      | ne      | ne      | ne           | ne  | ne  | ne    | ne  | ne  |
| PB <sup>a</sup>  | ne                           | ne   | ne   | 23 416  | ne      | ne      | ne           | ne  | ne  | ne    | ne  | ne  |
| DEX <sup>a</sup> | ne                           | ne   | ne   | ne      | ne      | ne      | 311          | ne  | ne  | ne    | ne  | ne  |
| CF <sup>a</sup>  | ne                           | ne   | ne   | ne      | ne      | ne      | ne           | ne  | ne  | 574   | ne  | ne  |

From Murchison (2010)

CF, clofibrate; DEX, dexamethasone; 3-MC, 3-methylcholanthrene; ne, not evaluated; PB, phenobarbital; \*  $P < 0.05$

<sup>a</sup> Commercially available hepatic microsomes from 3-MC-, PB-, DEX- and CF-treated Sprague-Dawley rats, used as positive controls.

<sup>b</sup> Below limit of quantification.

Isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 93:7 *syn:anti*) was tested for its ability to act as an agonist of human estrogen receptor alpha (hER $\alpha$ ) using the hER $\alpha$ -HeLa-9903 cell line and luciferase as reporter gene. Cell viability was monitored by propidium iodide uptake, and precipitation was determined by a light-scattering procedure using nephelometry. The positive control was 17 $\beta$ -estradiol ( $10^{-9}$  mol/l).

Isopyrazam concentrations greater than  $10^{-5}$  mol/l were found to be cytotoxic and induce precipitation. Therefore, isopyrazam concentrations of  $10^{-5}$  mol/l and below were tested for transcriptional activation of hER $\alpha$ . No increase in luciferase activity was observed in this range of isopyrazam concentrations (Toole, 2011).

(c) *Rat uterotrophic assay*

Isopyrazam (batch No. SMU6AP001, purity 96.4%, 93:7 *syn:anti*) was administered for 3 consecutive days at a dose level of 300 mg/kg bw per day by gavage to groups of six 10-week-old ovariectomized (at week 6) female CrI:WI(Han) rats. As a positive control, 17 $\beta$ -ethinylestradiol at a dose of 0.03 mg/kg bw per day was used; as a negative control, dibutylphthalate at a dose of 1000 mg/kg bw per day was used. All animals were observed twice daily for mortality and moribundity. Clinical observations, body weights and feed consumption were recorded daily throughout the study. Animals were terminated on study day 3 (the day following the last dose administration), and a gross examination of the uterus was conducted; uterine weights (wet and blotted) were recorded.

Whereas 17 $\beta$ -ethinylestradiol induced a statistically significant increase in uterine weights by approximately 30%, no effect on uterine weights was observed for isopyrazam or dibutylphthalate (Kuhl, 2011b).

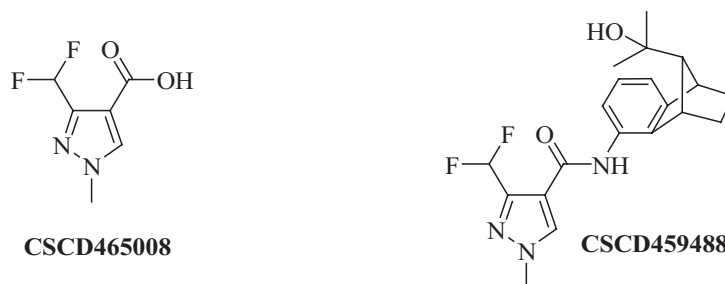
(d) *Studies with metabolites CSCD465008 and CSCD459488*

CSCD465008, a soil and plant metabolite, and CSCD459488, a soil, plant and aquatic metabolite and also identified in rat in vitro metabolism (White, 2008), were investigated in acute and short-term toxicity studies and genotoxicity studies. CSCD459488 was also investigated in a developmental toxicity study in rabbits. Their structures are illustrated in Figure 7.

*CSCD465008, a soil and plant metabolite of isopyrazam*

*Acute toxicity.* The acute oral toxicity of CSCD465008 (batch No. MES-103/1, purity  $94.0 \pm 2\%$ ) at 2000 mg/kg bw was tested in five female HanRCC Wistar rats. No mortalities were seen, but ruffled

**Figure 7. Structures of metabolites CSCD465008 and CSCD459488**



fur, slight sedation and hunched posture were observed within the first few hours after treatment. The  $LD_{50}$  of CSCD465008 in female rats was greater than 2000 mg/kg bw (Simon, 2008b).

*Short-term studies of toxicity.* CSCD465008 (batch No. MES-103/1, purity 94.0%) was administered for 28 days in the diet to groups of five male and five female 7-week-old Crl:WI(Han) rats. The concentrations in feed were 0, 2000, 6000 and 12 000 ppm (equal to 0, 175, 497 and 1018 mg/kg bw per day in males and 0, 176, 525 and 1107 mg/kg bw per day in females). Clinical observations were recorded daily, and body weights and feed consumption were recorded weekly. At week 3, all animals were subjected to a FOB, and locomotor activity was monitored within the detailed clinical observation. In week 3, all animals underwent an ophthalmic examination. At study termination, haematology, urinalysis and clinical chemistry were analysed. Organs of all animals were examined macroscopically and weighed. A range of tissues from control and high-dose animals was examined histologically. Livers and duodenum were investigated microscopically, and the EROD (CYP1A1/2) and PROD (CYP2B1/2) activities of liver microsomes were analysed. Hepatic microsomes from  $\beta$ -naphthoflavone-treated (100 mg/kg bw for 4 days) and phenobarbital-treated (80 mg/kg bw for 4 days) male and female Sprague-Dawley rats were used as positive controls.

None of the investigated parameters showed any treatment-related changes, and no induction of CYP1A or CYP2B activity was noted (Table 26). The NOAEL was 12 000 ppm (equal to 1018 mg/kg bw per day), the highest dose tested (Walraven, 2008).

*Genotoxicity in vitro.* CSCD465008 (batch No. MES-103/1, purity  $94 \pm 2\%$ ) was tested for genotoxicity in a range of guideline-compliant assays in vitro. No evidence for genotoxicity was observed in any test. The results are summarized in Table 27.

#### *CSCD459488, a soil, plant and aquatic metabolite of isopyrazam*

*Acute toxicity.* The acute oral toxicity of CSCD459488 (batch No. MES-111/3, purity 99%) at a dose of 2000 mg/kg bw was tested in five female HanRCC:WIST rats. No signs of toxicity were observed. The  $LD_{50}$  of CSCD465008 in female rats was greater than 2000 mg/kg bw (Simon, 2008a).

*Short-term studies of toxicity.* CSCD459488 (batch No. MES-111/3, purity 99%) was administered for 28 days in the diet to groups of five male and five female 7-week-old Crl:WI(Han) rats. The concentrations in feed were 0, 300, 4000 and 10 000 ppm (equal to 0, 27, 370 and 927 mg/kg bw per day in males and 0, 29, 388 and 906 mg/kg bw per day in females). Detailed clinical observations were recorded weekly, and body weights and feed consumption were recorded twice weekly. At week 3, all animals were subjected to a FOB, and locomotor activity was monitored within the detailed clinical observation. In week 3, all animals underwent an ophthalmic examination. At study termination, haematology, urinalysis and clinical chemistry were analysed. Organs of all animals were examined macroscopically and weighed. Organs from the control and high-dose groups and livers from all groups were investigated microscopically, and EROD (CYP1A1/2) and PROD (CYP2B1/2) activities of liver microsomes were analysed. Hepatic microsomes from 3-methylcholanthrene- and phenobarbital-treated

**Table 26. CYP1A and CYP2B activities in hepatic liver microsomes of male and female rats treated with CSCD465008**

| Dietary concentration (ppm) | Activity relative to control (fold change) |        |       |        |
|-----------------------------|--------------------------------------------|--------|-------|--------|
|                             | CYP1A                                      |        | CYP2B |        |
|                             | Male                                       | Female | Male  | Female |
| 2000                        | 0.9                                        | 0.9    | 1.1   | 1.0    |
| 6000                        | 0.7                                        | 0.8    | 0.9   | 0.9    |
| 12 000                      | 0.7                                        | 0.9    | 1.2   | 1.0    |
| Phenobarbital               | 2.8                                        | 2.8    | 23.1  | 53.8   |
| $\beta$ -Naphthoflavone     | 11.9                                       | 15.9   | 1.7   | 4.9    |

From Walraven (2008)

**Table 27. Results of genotoxicity studies with CSCD465008**

| End-point               | Test system                                                                                         | Concentration         | Lot No.; purity (%) | Result   | Reference            |
|-------------------------|-----------------------------------------------------------------------------------------------------|-----------------------|---------------------|----------|----------------------|
| Reverse mutation (Ames) | <i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537;<br><i>Escherichia coli</i> WP2, WP2 uvrA | 3–5000 $\mu$ g/plate  | MES-103/1; 94       | Negative | Sokolowski (2008b)   |
| Mouse lymphoma TK       | L5178Y cell line                                                                                    | 110–1760 $\mu$ g/ml   | MES-103/1; 94       | Negative | Wollny (2008b)       |
| Chromosomal aberration  | Human lymphocytes                                                                                   | 574.7–1760 $\mu$ g/ml | MES-103/1; 94       | Negative | Bohnenberger (2008b) |

TK, thymidine kinase

male and female Sprague-Dawley rats were used as positive controls; the dose levels and duration of treatment were not specified.

In males and females, the body weight–adjusted liver weights were increased by 4–6% at 300 ppm and statistically significantly by 25–33% and by 33–36% at 4000 ppm and 10 000 ppm, respectively. Centrilobular hepatocyte hypertrophy was observed in both sexes at 4000 and 10 000 ppm, but was more pronounced in males. In males at 4000 and 10 000 ppm, minimal follicular cell hypertrophy of the thyroid was noted. In males, total microsomal CYP content was approximately doubled in the 4000 and 10 000 ppm groups and only slightly elevated in females. CYP1A and CYP2B activities were statistically significantly (except males for CYP2B at 300 ppm) increased at all dose levels (Table 28). For CYP1A, the induction at 4000 ppm and above was more pronounced in males; for CYP2B, the induction was more pronounced in females at all dose levels.

The NOAEL was 300 ppm (equal to 27 mg/kg bw per day), based on liver weight changes at 4000 ppm (equal to 370 mg/kg bw per day) (Robertson, 2009).

**Developmental toxicity.** In a developmental toxicity study, CSCD459488 (batch No. MES 111/3, purity 99%) was administered by oral gavage from GD 7 to GD 28 to groups of 10 female mated 24-week-old New Zealand White rabbits. The dose levels were 0, 150, 500 and 1000 mg/kg bw per day. Clinical observations, body weights and feed consumption were recorded. All rabbits were terminated at GD 29, blood samples were taken for possible analysis of CSCD459488 and macroscopic examination of the uterus, uterine contents, positions of fetuses in the uterus and the number of corpora lutea was performed. Livers were weighed and samples retained for possible microscopic examination. The uteri (and contents) with live fetuses were weighed. Fetuses were weighed individually and examined for gross external, visceral and skeletal abnormalities.

**Table 28. CYP1A and CYP2B activities in liver microsomes of male and female rats treated with CSCD459488**

| Dietary concentration (ppm)       | Activity relative to control (fold change) |        |          |         |
|-----------------------------------|--------------------------------------------|--------|----------|---------|
|                                   | CYP1A                                      |        | CYP2B    |         |
|                                   | Male                                       | Female | Male     | Female  |
| 300                               | 1.4***                                     | 1.9*   | 2.7      | 54.0*** |
| 4000                              | 3.7**                                      | 1.8*   | 100.2*** | 117.4*  |
| 10 000                            | 4.2***                                     | 2.3*   | 124.6*   | 78.7*** |
| Phenobarbital <sup>a</sup>        | —                                          | —      | 119.7    | —       |
| 3-Methylcholanthrene <sup>a</sup> | 7.6                                        | —      | —        | —       |

From Robertson (2009)

— not tested; \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.005$

<sup>a</sup> No treatment details provided.

The body weight development of dams was not affected by treatment. At all dose levels, maternal liver weights were increased dose relatedly, by 17.5%, 31.8% and 55.1% at 150, 500 and 1000 mg/kg bw per day, respectively. In the high-dose group, late resorptions per litter (5.6%) were statistically significantly increased, primarily due to three resorptions in one female compared with controls (0%). One high-dose fetus had a narrow pectoral region, macroglossia and enlarged lungs and larynx. This very low incidence is not considered to be treatment related in the absence of any other malformation.

The maternal NOAEL was 150 mg/kg bw per day, based on significant liver weight increases greater than 20% at higher doses. The NOAEL for developmental toxicity was 1000 mg/kg bw per day, the highest dose tested (Sawhney Coder, 2008a).

*Genotoxicity in vitro.* CSCD459488 (batch No. TE-6472/4 or batch No. MES 111/3, purity 99%) was tested for genotoxicity in a range of guideline-compliant assays in vitro. No evidence for genotoxicity was observed in any test. The results are summarized in Table 29.

*Induction of enzymes and DNA synthesis.* CSCD459488 (SYN545364, batch No. MES 111/4, purity 96%) was tested for its ability to induce CYP2B (PROD) and CYP2B/3A (BROD) activities and cell proliferation (measured as the change in DNA synthesis [S-phase of the cell cycle]) in isolated primary hepatocytes of female Han Wistar rats. Phenobarbital sodium salt was used as positive control. Hepatocytes were exposed to CSCD459488 at 1, 3, 10, 30, 100 and 500  $\mu\text{mol/l}$  and phenobarbital sodium salt at 10, 100 and 1000  $\mu\text{mol/l}$  or vehicle (0.5% DMSO) for 96 hours. Cytotoxicity was assayed by measuring the change in cellular ATP levels.

Both phenobarbital sodium salt and CSCD459488 induced PROD and BROD activities and DNA synthesis (Table 30). Comparing the potencies using the per cent changes at the lowest doses producing a statistically significant effect gives a higher potency of CSCD459488 compared with phenobarbital sodium salt, for PROD 52-fold, for BROD 86-fold and for DNA synthesis 10-fold (Elcombe, 2011b).

CSCD459488 (SYN545364, batch No. MES 111/4, purity 96%) was tested for its ability to induce CYP2B (PROD) and CYP2B/3A (BROD) activities and cell proliferation (measured as the change in DNA synthesis [S-phase of the cell cycle]) in isolated primary female human hepatocytes. Phenobarbital sodium salt was used as positive control. Hepatocytes were exposed to CSCD459488 at 1, 3, 10, 30, 65 and 100  $\mu\text{mol/l}$  and phenobarbital sodium salt at 10, 100 and 1000  $\mu\text{mol/l}$  or vehicle (0.5% DMSO) for 96 hours. Cytotoxicity was assayed by measuring the change in cellular ATP levels.

Phenobarbital sodium salt and CSCD459488 did not induce DNA synthesis statistically significantly at any concentration level. Phenobarbital sodium salt induced PROD and BROD activities at

**Table 29. Results of genotoxicity studies with CSCD459488**

| End-point               | Test system                                                                                             | Concentration    | Lot No.; purity (%) | Result   | Reference            |
|-------------------------|---------------------------------------------------------------------------------------------------------|------------------|---------------------|----------|----------------------|
| Reverse mutation (Ames) | <i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537; <i>Escherichia coli</i> WP2, WP2 <i>uvrA</i> | 3–5000 µg/plate  | TE-6472/4; 99       | Negative | Sokolowski (2008a)   |
| Mouse lymphoma TK       | L5178Y cell line                                                                                        | 25–800 µg/ml     | MES 111/3           | Negative | Wollny (2008a)       |
| Chromosomal aberration  | Human lymphocytes                                                                                       | 31.8–522.4 µg/ml | MES 111/3           | Negative | Bohnenberger (2008a) |

TK, thymidine kinase

10 and 100 µmol/l, respectively, statistically significantly and CSCD459488 at 1 and 3 µmol/l (Table 31). Comparing the potencies using the per cent changes at the lowest doses producing a statistically significant effect gives a 9-fold higher potency of CSCD459488 compared with phenobarbital sodium salt for PROD activity and a 30-fold higher potency for BROD activity (Elcombe, 2011a).

CSCD459488 (batch No. MES111/4, purity 96%) was administered for 3 consecutive days at a dose level of 300 or 1000 mg/kg bw per day by gavage to groups of six 9-week-old ovariectomized (at week 6) female CrI:WI(Han) rats. As a positive control, 17β-ethinylestradiol at a dose of 0.03 mg/kg bw per day was used, and as a negative control, dibutylphthalate at a dose of 1000 mg/kg bw per day was used. All animals were observed twice daily for mortality and moribundity. Clinical observations, body weights and feed consumption were recorded daily throughout the study. Animals were terminated on study day 3 (the day following the last dose administration), and a gross examination of the uterus was conducted; uterine weights (wet and blotted) were recorded.

Whereas 17β-ethinylestradiol induced a statistically significant increase in uterine weights by approximately 300% (17β-ethinylestradiol was used at a dose 10-fold higher than in the uterotrophic assay with isopyrazam), no effect on uterine weights was observed for CSCD459488 or dibutylphthalate (Kuhl, 2011a).

### 3. Observations in humans

No reports were submitted.

## Comments

### Biochemical aspects

Isopyrazam administered to rats at 1 or 75 mg/kg bw was rapidly absorbed, with peak levels of radiolabel occurring in plasma within 3–6 hours post-dosing. Approximately 70% of the dose was absorbed at 1 and 75 mg/kg bw. The terminal half-life of the labelled material was 5–9 hours. No saturation of absorption was observed, but the area under the curve increased disproportionately with dose, indicating saturation of elimination at higher doses. In the low-dose group, 91–97% of the absorbed dose was excreted within 48 hours after administration. Highest residues were identified in the liver, kidney, thyroid and adrenals. The major route of excretion was by bile, accounting for approximately 65–90% of the absorbed dose, with the rest being excreted in urine within 48 hours. After repeated dosing, no accumulation of radioactivity in rats was observed. There were no significant differences between the toxicokinetic parameters of the two diastereoisomers *syn* and *anti*. The predominant metabolic pathway for isopyrazam and its *N*-demethylated metabolite is hydroxylation in

**Table 30. Changes in enzyme induction and DNA synthesis in cultured primary female rat hepatocytes**

| Treatment                  | % change                  |               |                |                |                |                |                |                |                          |              |
|----------------------------|---------------------------|---------------|----------------|----------------|----------------|----------------|----------------|----------------|--------------------------|--------------|
| Control                    | Phenobarbital sodium salt |               |                |                |                |                |                |                |                          |              |
|                            | CSCD459488                |               |                |                |                |                |                |                |                          |              |
|                            | Concentration (μmol/l)    |               |                |                |                |                |                |                |                          |              |
|                            | 0                         | 10            | 100            | 1000           | 1              | 3              | 10             | 30             | 100                      | 500          |
| ATP <sup>a</sup>           | —                         | 99.1 ± 5.2    | 101.8 ± 5.2    | 132.1 ± 7.2**  | 113.8 ± 6.1**  | 115.5 ± 5.2**  | 111.4 ± 7.1*   | 121.7 ± 5.3**  | 129.3 ± 4.1**            | 2.3 ± 0.2**  |
| DNA synthesis <sup>b</sup> | —                         | 136.8 ± 30.6* | 181.8 ± 12.4** | 185.3 ± 12.9** | 136.9 ± 21.7*  | 176.5 ± 27.9** | 168.0 ± 9.4**  | 162.3 ± 16.9** | 81.3 ± 19.5 <sup>c</sup> | <sup>d</sup> |
| PROD <sup>c</sup>          | —                         | 91.4 ± 27.9   | 497.5 ± 57.5** | 567.5 ± 59.7** | 257.3 ± 16.8** | 424.2 ± 42.6** | 527.8 ± 35.3** | 561.7 ± 37.4** | 69.5 ± 30.8 <sup>c</sup> | <sup>d</sup> |
| BROD <sup>c</sup>          | —                         | 138.6 ± 30.7  | 347.7 ± 71.6** | 456.7 ± 48.7** | 297.4 ± 20.7** | 392.3 ± 77.4** | 400.8 ± 67.5** | 342.9 ± 4.2**  | 108.5 ± 7.3 <sup>c</sup> | <sup>d</sup> |

From Elcombe (2011b)

ATP, adenosine-5'-triphosphate; BROD, benzyloxyresorufin *O*-debenzylase; DNA, deoxyribonucleic acid; PROD, 7-pentoxoresorufin *O*-dealkylase; \*  $P < 0.05$ ; \*\*  $P < 0.01$ <sup>a</sup>  $n = 6$  per group.<sup>b</sup>  $n = 5$  per group.<sup>c</sup> No statistical analyses performed (visible cytotoxicity).<sup>d</sup> No analyses performed (excessive cytotoxicity).<sup>e</sup>  $n = 3$  per group.**Table 31. Changes in enzyme induction and DNA synthesis in cultured primary human female hepatocytes**

| Treatment                  | % change relative to control |                           |               |                |               |                |               |                |                         |             |
|----------------------------|------------------------------|---------------------------|---------------|----------------|---------------|----------------|---------------|----------------|-------------------------|-------------|
|                            | Control                      | Phenobarbital sodium salt |               |                |               |                |               |                |                         |             |
|                            | CSCD459488                   |                           |               |                |               |                |               |                |                         |             |
| Concentration (μmol/l)     |                              |                           |               |                |               |                |               |                |                         |             |
|                            | 0                            | 10                        | 100           | 1000           | 1             | 3              | 10            | 30             | 100                     | 500         |
| ATP <sup>a</sup>           | —                            | 78.5 ± 15.3*              | 87.0 ± 8.6    | 78.0 ± 8.0*    | 79.0 ± 5.9    | 86.3 ± 3.2     | 90.1 ± 7.4    | 79.2 ± 1.8*    | 28.7 ± 1.8**            | 0.7 ± 0.1** |
| DNA synthesis <sup>b</sup> | —                            | 84.9 ± 24.3               | 102.2 ± 40.9  | 91.3 ± 22.3    | 92.6 ± 27.5*  | 130.2 ± 35.2   | 120.2 ± 52.6  | 65.7 ± 12.5    | c                       | c           |
| PROD <sup>d</sup>          | —                            | 241.2 ± 28.0**            | 235.4 ± 57.3* | 623.8 ± 218.2* | 218.6 ± 54.5* | 201.4 ± 30.4** | 187.0 ± 107.0 | 222.0 ± 48.7*  | c                       | c           |
| BROD <sup>d</sup>          | —                            | 133.7 ± 19.8              | 221.5 ± 34.4* | 555.6 ± 65.5** | 120.4 ± 19.5  | 200.4 ± 34.5*  | 232.4 ± 113.6 | 496.7 ± 91.0** | 39.1 ± 9.1 <sup>c</sup> | c           |

From Elcombe (2011a)

ATP, adenosine-5'-triphosphate; BROD, benzyloxyresorufin *O*-debenzylase; DNA, deoxyribonucleic acid; PROD, 7-pentoxoresorufin *O*-dealkylase; \*  $P < 0.05$ ; \*\*  $P < 0.01$ <sup>a</sup>  $n = 6$  per group.<sup>b</sup>  $n = 5$  per group.<sup>c</sup> No analyses performed (excessive cytotoxicity).<sup>d</sup>  $n = 3$  per group.<sup>e</sup> No statistical analyses performed (excessive cytotoxicity).

the bicyclic isopropyl moiety, followed by further oxidation to form the carboxylic acid or to give rise to multiple hydroxyl moieties, with subsequent formation of glucuronic acid or sulfate conjugates. The structure of isopyrazam provides the potential for stereoisomerization of most metabolites.

### Toxicological data

Isopyrazam technical with a *syn:anti* ratio of up to 69.7:30.3 is of low acute oral toxicity. The oral LD<sub>50</sub> was greater than 2000 mg/kg bw in female rats. However, as the oral LD<sub>50</sub> of pure *anti* isomer or of a 1:1 *syn:anti* batch was 310.2 mg/kg bw in female rats, there seems to be an isomeric difference in toxicity at very high dose levels. By dermal application, the LD<sub>50</sub> of a 92.8:7.2 *syn:anti* batch was greater than 5000 mg/kg bw, and the LC<sub>50</sub> in an inhalation study was greater than 5.28 mg/l. Isopyrazam was not irritating to the skin and only initially slightly irritating to the eye. Isopyrazam showed skin sensitizing potential in a mouse local lymph node assay.

In repeated-dose studies in mice, rats and dogs, the main effects were changes in clinical chemistry (plasma protein, cholesterol, triglycerides, liver enzymes) and haematological parameters (red blood cell counts, haemoglobin, haematocrit), effects on the liver (hepatocellular hypertrophy) and body weight changes.

In a 13-week mouse feeding study with dietary concentrations up to 7000 ppm, reduced body weight in spite of higher feed consumption was observed. Red blood cell parameters were reduced and platelet counts were increased, particularly at the high dose. In females, a few clinical chemistry parameters were elevated. Relative liver weights were increased, and hepatocellular hypertrophy was observed. For one male and one female high-dose animal, necrotic liver nodules were noted. The NOAEL was 2500 ppm (equal to 390.8 mg/kg bw per day), based on haematological changes at 7000 ppm (equal to 1328.8 mg/kg bw per day).

In a study to compare the toxicity of the *syn* and *anti* conformation isomers of isopyrazam, pure *syn* epimer, pure *anti* epimer and 1:1 *syn:anti* epimer were administered to rats at up to 5000 ppm for 4 weeks in the diet. With pure *anti* and 1:1 *syn:anti*, feed intake and body weight were reduced. Haematological and clinical chemistry parameters were affected with all three compounds, but more severely with pure *anti* and 1:1 *syn:anti*. Liver weights were increased in females in all dosed groups and in males at 2000 ppm and above. The Meeting considered the liver findings as adaptive effects and not toxicologically relevant. All compounds in both sexes mildly elevated the total hepatic CYP content and mildly increased EROD (CYP1A) activity, but markedly increased PROD (CYP2B) activity. The NOAEL for pure *syn* was 2000 ppm (equal to 176.7 mg/kg bw per day), based on reduced body weight and increased cholesterol levels at 5000 ppm (equal to 437.3 mg/kg bw per day). The NOAEL for pure *anti* and 1:1 *syn:anti* was 500 ppm (equal to 45.2 mg/kg bw per day), based on reduced body weight gain and increased cholesterol levels at 2000 ppm (equal to 176.7 mg/kg bw per day). In two further 4-week rat feeding studies with slightly different isopyrazam batches (*syn:anti* ratios of 89:11 and 92.8:7.2), the NOAELs were 500 ppm (equal to 46.1 mg/kg bw per day) and 300 ppm (equal to 28.1 mg/kg bw per day), based on body weight changes, clinical chemistry and haematological changes at 2000 ppm (equal to 174.9 mg/kg bw per day) and above.

In a 13-week rat feeding study with dietary concentrations of isopyrazam (92.8:7.2 *syn:anti*) of up to 6000 ppm, reduced feed consumption and body weight gain were noted. Relative liver weight increases were accompanied by hepatocellular hypertrophy. Relative brain weights were decreased in both sexes at the highest dose level. Triglyceride and bilirubin levels were decreased at 1500 ppm and above. The NOAEL was 300 ppm (equal to 21.3 mg/kg bw per day), based on clinical chemistry changes at 1500 ppm (equal to 106.3 mg/kg bw per day). In a 13-week rat feeding study with dietary concentrations of isopyrazam (97.7:2.3 *syn:anti*) of up to 4000 ppm, the NOAEL was 1000 ppm (equal to 74.1 mg/kg bw per day), based on significantly increased relative liver weights and hepatocellular hypertrophy in males and females at 4000 ppm (equal to 314.8 mg/kg bw per day).

In a comparative 13-week rat feeding study with dietary concentrations of two batches of isopyrazam (*syn:anti* ratios 92.8:7.2 and 69.7:30.3) up to 2000 ppm, the NOAEL for both compounds was 250 ppm (equal to 20.5 mg/kg bw per day), based on body weight effects and hepatocellular hypertrophy and vacuolation at 2000 ppm (equal to 161.0 mg/kg bw per day).

In dogs, two 13-week gelatine capsule gavage studies were performed with two batches of isopyrazam (*syn:anti* ratios 92.8:7.2 and 69.7:30.3). In the first study with isopyrazam *syn:anti* 92.8:7.2, several behavioural changes, reduced feed consumption and body weight gain, changes in clinical chemistry parameters and increases in liver weights were noted. The NOAEL in this study was 30 mg/kg bw per day, based on behavioural changes and liver weight increases at 100 mg/kg bw per day. In the second study with isopyrazam *syn:anti* 69.7:30.3, the NOAEL was 30 mg/kg bw per day, based on clinical observations and initial body weight loss at 250 mg/kg bw per day. In a 52-week gelatine capsule gavage study with isopyrazam (*syn:anti* 92.8:7.2), no clinical signs were observed. Initially reduced feed consumption and lower body weights were noted throughout the study. Some clinical chemistry parameters showed occasional modest changes but were without any histopathological correlates or other signs of toxicity. The NOAEL in this study was 25 mg/kg bw per day, based on changes in clinical chemistry parameters and in liver weight at higher dose levels. The overall NOAEL for the effects of isopyrazam with a *syn:anti* ratio down to 69.7:30.3 in the 3-month and 1-year studies in dogs was 30 mg/kg bw per day.

In an 18-month feeding study in mice with dietary concentrations of isopyrazam (*syn:anti* 92.8:7.2) up to 3500 ppm, the incidence of males with eye discharge was elevated at 3500 ppm. Also at 3500 ppm, body weight gain was reduced in both sexes, body weight-adjusted spleen weights were decreased and liver weights were increased. At 500 ppm, the incidence of periportal hepatocellular hypertrophy in females was increased. At 3500 ppm, the incidences of epithelial eosinophilic droplets in the nasal cavity of males and in the gall bladder of females were elevated. The incidences of benign or malignant tumours were not increased at any dose. The NOAEL was 70 ppm (equal to 9.9 mg/kg bw per day), based on periportal hepatocellular hypertrophy in females at 500 ppm (equal to 74.9 mg/kg bw per day).

Isopyrazam was not carcinogenic in mice.

In a 104-week feeding study in rats with dietary concentrations up to 3000 ppm isopyrazam (*syn:anti* 92.8:7.2), body weight gain was decreased in all dosed females and in high-dose males. In both sexes, haematological parameters were changed and the prothrombin time was reduced, and in females, the activated partial thromboplastin time was prolonged at 500 ppm and above. There were changes in some clinical chemistry parameters (e.g. urea, triglyceride and bilirubin levels in females) in all dose groups. At terminal kill, relative brain weights in females at 3000 ppm were increased, and liver weights in both sexes were increased at 500 and 3000 ppm. Adrenal weights in females were decreased at 3000 ppm. The incidences of foci of eosinophilic hepatocytes were increased statistically significantly at 500 ppm and above in both sexes. The hepatocellular pigmentation in all dosed females and in high-dose males was not considered to be of toxicological relevance at the lowest dose because it was of minimal severity. The increased centrilobular hepatocellular hypertrophy observed at all dose levels in both sexes was considered to represent adaptive changes and not to be of toxicological significance. In females at 3000 ppm, there was an increase in hepatocellular adenoma (17%), and at 3000 ppm, one hepatocellular carcinoma was found in each sex. In the high-dose females, the incidence of uterine endometrial adenocarcinoma was increased (23%). The NOAEL was 100 ppm (equal to 5.5 mg/kg bw per day), based on reduced body weight gain in females and increased incidences of foci of eosinophilic hepatocytes and clinical chemistry changes of equivocal toxicological significance in both sexes at 500 ppm (equal to 27.6 mg/kg bw per day).

Isopyrazam was carcinogenic in female rats at the highest dose tested.

The potential genotoxicity of isopyrazam was tested in an adequate range of in vitro and in vivo studies, providing no evidence of genotoxic potential.

The Meeting concluded that isopyrazam is unlikely to be genotoxic.

In mechanistic studies to evaluate possible modes of action for liver tumours in female rats, isopyrazam was shown to induce CYP2B and CYP3A activities and replicative deoxyribonucleic acid (DNA) synthesis in female rat hepatocytes in vitro with a significantly higher potency than phenobarbital. CYP3A was also induced with a high potency in female human hepatocytes in vitro, whereas phenobarbital showed a weak induction potential. In a 14-day feeding study in rats, CYP1A- and CYP2B-dependent activities were induced significantly at all three time points (3, 8 and 15 days after treatment with 500 or 3000 ppm), whereas CYP3A- and CYP4A-dependent activities were not induced. The significant in vivo CYP1A induction and the very high potency CYP2B (in vitro and in vivo) and CYP3A induction (in vitro) for isopyrazam suggest more than phenobarbital-like enzyme induction. Although microsomal enzyme induction was observed, no clear mode of action was identified that could be causally linked to the liver adenoma in female rats.

In an in vitro test with hER $\alpha$ , isopyrazam did not show significant binding capacity. Isopyrazam was negative in a rat uterotrophic assay. Therefore, an estrogen-like mode of action as a possible explanation for the uterine endometrial adenocarcinoma is not supported.

On the basis of the absence of genotoxicity and the absence of carcinogenicity in mice and the fact that an increase in the incidences of hepatocellular adenoma and uterine endometrial adenocarcinoma in female rats occurred only at the highest dose tested, the Meeting concluded that isopyrazam is unlikely to pose a carcinogenic risk to humans at dietary exposure levels.

In a two-generation study of reproductive toxicity in rats at dietary concentrations up to 3000 ppm isopyrazam (*syn:anti* 92.8:7.2), F<sub>0</sub> and F<sub>1</sub> rats had decreased body weight at 500 and 3000 ppm. Hepatocellular hypertrophy and increases in liver weights were noted in F<sub>0</sub> and F<sub>1</sub> animals at 500 ppm and above. In F<sub>0</sub> males at 500 ppm and above, thyroid weights were increased statistically significantly. In F<sub>0</sub> and F<sub>1</sub> females at 3000 ppm, weights of the uterus with cervix were decreased statistically significantly. Kidney weights in F<sub>1</sub> animals were dose-relatedly increased at all dose levels in both sexes, statistically significantly in females at all dose levels and in males at 3000 ppm. Ovary weights in high-dose F<sub>0</sub> and F<sub>1</sub> females were statistically significantly decreased. F<sub>1A</sub> and F<sub>2A</sub> pup body weights were reduced at 3000 ppm. At 500 ppm and above, mean total litter weights were reduced. F<sub>1</sub> males at 3000 ppm showed statistically significantly delayed preputial (2.3 days) separation, and F<sub>1</sub> females at 3000 ppm showed statistically significantly delayed vaginal opening (2 days). Whereas the males showed statistically significantly reduced body weights (–7%), the body weights of females were unchanged. The NOAEL for parental toxicity was 100 ppm (equal to 8.1 mg/kg bw per day), based on decreased body weight gain and organ weight changes at 500 ppm (equal to 40.6 mg/kg bw per day) in parental F<sub>0</sub> and F<sub>1</sub> animals. The NOAEL for postnatal developmental toxicity was 100 ppm (equal to 8.1 mg/kg bw per day), based on decreased mean total litter weights at 500 ppm (equal to 40.6 mg/kg bw per day). The NOAEL for reproductive performance was 3000 ppm (equal to 239.1 mg/kg bw per day), the highest dose tested.

In a study on the developmental toxicity of isopyrazam (*syn:anti* 92.8:7.2) in rats at dose levels up to 250 mg/kg bw per day administered by gavage, two high-dose dams were killed in extremis on GDs 20 and 21 because they were showing severe signs of toxicity. The high dose group animals showed reduced feed consumption and reduced body weight, and fetal body weights were decreased in this group. In the 250 mg/kg bw per day group, one fetus with hydroencephalus and microphthalmia was observed, and in another litter, a fetus with hydrocephalus only was noted. Non-ossified cervical centra and incomplete xiphoid cartilage were noted at 75 mg/kg bw per day and above. The NOAEL for maternal toxicity was 75 mg/kg bw per day, based on reduced body weight and clinical signs of toxicity at 250 mg/kg bw per day. The NOAEL for developmental toxicity was 20 mg/kg bw per day, based on non-ossified cervical centra at 75 mg/kg bw per day.

In a study on the developmental toxicity of isopyrazam (*syn:anti* 69.7:30.3) in rats at dose levels up to 200 mg/kg bw per day administered by gavage, ventral recumbency and sedation were noted

in all dams at 200 mg/kg bw per day, from the 1st day of treatment (GD 4) throughout the 1st week. Feed consumption and body weight gain were reduced from GD 4 in animals at 75 mg/kg bw per day and above. At 75 mg/kg bw per day and above, fetal body weights were lower than those of controls. One fetus at 200 mg/kg bw per day was found with diaphragmatic hernia. Increased incidences of delayed or absent ossification of cervical vertebral bodies were observed at 200 mg/kg bw per day and of incompletely ossified sternebrae at 75 mg/kg bw per day and above. Additionally, non-ossified structures in forelimbs and hindlimbs were identified. The NOAEL for maternal and developmental toxicity was 20 mg/kg bw per day, based on clinical signs and reduced body weight gain in dams and lower fetal body weights at 75 mg/kg bw per day.

In two range-finding studies on the developmental toxicity of isopyrazam (*syn:anti* 92.8:7.2) in Himalayan rabbits at dose levels up to 1000 mg/kg bw per day, no maternal toxicity was observed. In fetuses, the incidences of small eyes, malrotated and flexed limbs and changes in the skull were increased at 400 mg/kg bw per day and above.

In a third range-finding study on the developmental toxicity of isopyrazam (*syn:anti* 92.8:7.2) in New Zealand White rabbits at dose levels up to 1000 mg/kg bw per day, maternal toxicity was noted at all dose levels from 400 to 1000 mg/kg bw per day, as dams had decreased body weight, increased relative liver weights, hepatocellular hypertrophy and changes in clinical chemistry parameters. At the high dose, fetal body weight was reduced and early resorptions were increased. Small eyes were noted in fetuses at 1000 mg/kg bw per day. Furthermore, absent gall bladders, extra papillary muscle in the heart and variations of major blood vessels were observed at all dose levels.

In a definitive study on the developmental toxicity of isopyrazam (*syn:anti* 92.8:7.2) in New Zealand White rabbits at dose levels up to 500 mg/kg bw per day, hepatocellular vacuolation was observed at 500 mg/kg bw per day. One fetus in the 500 mg/kg bw per day group had bilateral microphthalmia. The NOAEL for maternal toxicity was 150 mg/kg bw per day, based on a statistically significantly increased incidence of centrilobular vacuolation at 500 mg/kg bw per day. The developmental NOAEL was 150 mg/kg bw per day, based on a single observation of bilateral microphthalmia at 500 mg/kg bw per day.

A low incidence of microphthalmia was consistently observed in dose range-finding and main studies in two different rabbit strains. Microphthalmia is a very rare finding in the rabbit strains used. Thus, the Meeting concluded that the low incidences of microphthalmia in treated rabbits could not be discounted.

The Meeting concluded that isopyrazam was teratogenic in rabbits.

In an acute neurotoxicity study in rats administered isopyrazam (*syn:anti* 92.8:7.2) at doses ranging from 30 to 2000 mg/kg bw, nonspecific and transient effects were apparent within 3 hours after dosing in all dose groups, with a dose-dependent increase in the incidence and severity of rigidity. The NOAEL for systemic toxicity was 30 mg/kg bw, based on clinical signs of toxicity at 250 mg/kg bw. The NOAEL for acute neurotoxicity was 2000 mg/kg bw, the highest dose tested.

In a 13-week rat feeding study of the neurotoxicity of isopyrazam (*syn:anti* 92.8:7.2) with dietary concentrations up to 6000 ppm, no behavioural or histological evidence for neurotoxicity was observed. The NOAEL for systemic toxicity was 1500 ppm (equal to 98.01 mg/kg bw per day), based on decreased body weight gain in females at 6000 ppm (equal to 382.26 mg/kg bw per day). The NOAEL for subchronic neurotoxicity was 6000 ppm (equal to 382.26 mg/kg bw per day), the highest dose tested.

### Toxicological data on metabolites

CSCD465008, a soil and plant metabolite, and CSCD459488, a rat, soil, plant and aquatic metabolite, were investigated in acute and short-term toxicity studies and an adequate range of in

vitro genotoxicity studies. CSCD459488 was also investigated in a developmental toxicity study in rabbits.

CSCD465008 and CSCD459488 were both of low acute oral toxicity, with LD<sub>50</sub> values greater than 2000 mg/kg bw, and did not give any evidence of genotoxic potential.

In a 4-week rat feeding study with dietary concentrations of CSCD465008 up to 12 000 ppm, no evidence for toxicity or for induction of EROD or PROD activity was observed. The NOAEL was 12 000 ppm (equal to 1018 mg/kg bw per day), the highest dose tested.

In a 4-week rat feeding study with dietary concentrations of CSCD459488 up to 10 000 ppm, the relative liver weights were increased at 300 ppm and above, and increased incidences of centrilobular hepatocyte hypertrophy and follicular cell hypertrophy of the thyroid were noted at 4000 ppm and above. Total hepatic microsomal CYP content was approximately doubled in males in the 4000 and 10 000 ppm groups and only slightly elevated in females in the same dose groups. EROD and PROD activities were statistically significantly increased at all dose levels. The NOAEL was 300 ppm (equal to 27 mg/kg bw per day), based on liver weight changes greater than 10% at higher doses.

In a study on the developmental toxicity of CSCD459488 in New Zealand White rabbits at dose levels up to 1000 mg/kg bw per day, relative maternal liver weights were increased at all dose levels. In the high-dose group, late resorptions per litter were increased primarily due to three resorptions in one female. The maternal NOAEL was 150 mg/kg bw per day, based on significant liver weight increases (> 20%) at higher dose levels. The NOAEL for developmental toxicity was 1000 mg/kg bw per day, the highest dose tested.

No reports on exposure of personnel working with isopyrazam were submitted.

The Meeting concluded that the existing database on isopyrazam was adequate to characterize the potential hazard to fetuses, infants and children.

### **Toxicological evaluation**

The Meeting established an acceptable daily intake (ADI) of 0–0.06 mg/kg bw, derived from the NOAEL of 5.5 mg/kg bw per day in the 104-week rat feeding study on the basis of decreased body weight gain in females and increased incidences of foci of eosinophilic hepatocytes and clinical chemistry changes (triglycerides, bilirubin) of equivocal toxicological significance in both sexes at 27.6 mg/kg bw per day. A safety factor of 100 was applied. The ADI is supported by the NOAEL of 9.9 mg/kg bw per day in the mouse 80-week feeding study, based on periportal hepatocellular hypertrophy in females at 56.2 mg/kg bw per day. The margin between the maximum ADI and the lowest-observed-adverse-effect level (LOAEL) at 232.8 mg/kg bw per day for uterine and liver tumours in female rats is approximately 3900.

The Meeting established an acute reference dose (ARfD) of 0.3 mg/kg bw, derived from the NOAEL of 30 mg/kg bw in the rat acute neurotoxicity study, on the basis of nonspecific clinical signs of toxicity (weak appearance and decreased activity) at 250 mg/kg bw. A safety factor of 100 was applied. In a rat developmental toxicity study, the NOAEL of 20 mg/kg bw per day for maternal and developmental toxicity was based on reduced body weight gain in dams only on day 4 of treatment. The margin between the ARfD and the LOAEL at 500 mg/kg bw per day for teratogenic effects (microphthalmia) in rabbits is approximately 1700.

***Levels relevant to risk assessment***

| Species | Study                                                             | Effect                    | NOAEL                                                  | LOAEL                                     |
|---------|-------------------------------------------------------------------|---------------------------|--------------------------------------------------------|-------------------------------------------|
| Mouse   | Eighteen-month study of toxicity and carcinogenicity <sup>a</sup> | Toxicity                  | 70 ppm, equal to 9.9 mg/kg bw per day                  | 500 ppm, equal to 56.2 mg/kg bw per day   |
|         |                                                                   | Carcinogenicity           | 3500 ppm, equal to 432.6 mg/kg bw per day <sup>b</sup> | —                                         |
| Rat     | Acute neurotoxicity <sup>c</sup>                                  | Toxicity                  | 30 mg/kg bw                                            | 250 mg/kg bw                              |
|         | Two-year study of toxicity and carcinogenicity <sup>a</sup>       | Toxicity                  | 100 ppm, equal to 5.5 mg/kg bw per day                 | 500 ppm, equal to 27.6 mg/kg bw per day   |
|         |                                                                   | Carcinogenicity           | 500 ppm, equal to 34.9 mg/kg bw per day                | 3000 ppm, equal to 232.8 mg/kg bw per day |
|         | Two-generation study of reproductive toxicity <sup>a</sup>        | Reproductive toxicity     | 3000 ppm, equal to 239.1 mg/kg bw per day <sup>b</sup> | —                                         |
|         |                                                                   | Parental toxicity         | 100 ppm, equal to 8.1 mg/kg bw per day                 | 500 ppm, equal to 40.6 mg/kg bw per day   |
|         |                                                                   | Offspring toxicity        | 100 ppm, equal to 8.1 mg/kg bw per day                 | 500 ppm, equal to 40.6 mg/kg bw per day   |
|         | Developmental toxicity study <sup>c</sup>                         | Maternal toxicity         | 20 mg/kg bw per day                                    | 75 mg/kg bw per day                       |
|         |                                                                   | Embryo and fetal toxicity | 20 mg/kg bw per day                                    | 75 mg/kg bw per day                       |
| Rabbit  | Developmental toxicity study <sup>c</sup>                         | Maternal toxicity         | 150 mg/kg bw per day                                   | 500 mg/kg bw per day                      |
|         |                                                                   | Embryo and fetal toxicity | 150 mg/kg bw per day                                   | 500 mg/kg bw per day                      |
| Dog     | Thirteen-week and 1-year studies of toxicity <sup>c,d</sup>       | Toxicity                  | 30 mg/kg bw per day                                    | 100 mg/kg bw per day                      |

<sup>a</sup> Dietary administration.<sup>b</sup> Highest dose tested.<sup>c</sup> Gavage administration.<sup>d</sup> Three studies combined.***Estimate of acceptable daily intake for humans***

0–0.06 mg/kg bw

***Estimate of acute reference dose***

0.3 mg/kg bw

***Information that would be useful for the continued evaluation of the compound***

Results from epidemiological, occupational health and other such observational studies of human exposures

***Critical end-points for setting guidance values for exposure to isopyrazam******Absorption, distribution, excretion and metabolism in mammals***

|                                    |                                    |
|------------------------------------|------------------------------------|
| Rate and extent of oral absorption | Rapid, 70%                         |
| Dermal absorption                  | No data                            |
| Distribution                       | Extensive, highest levels in liver |

|                                                                             |                                                                     |
|-----------------------------------------------------------------------------|---------------------------------------------------------------------|
| Potential for accumulation                                                  | Low, no evidence of accumulation                                    |
| Rate and extent of excretion                                                | Rapid, close to 100% within 48 h, mainly via bile                   |
| Metabolism in animals                                                       | Extensive, primarily via hydroxylation at bicyclic-isopropyl moiety |
| Toxicologically significant compounds (animals, plants and the environment) | Isopyrazam, CSCD459488                                              |

### *Acute toxicity*

|                                                    |                                                                                                            |
|----------------------------------------------------|------------------------------------------------------------------------------------------------------------|
| Rat, LD <sub>50</sub> , oral                       | > 2000 mg/kg bw (69.7:30.3 <i>syn:anti</i> )<br>310.2 mg/kg bw (1:1 <i>syn:anti</i> and pure <i>anti</i> ) |
| Rat, LD <sub>50</sub> , dermal                     | > 5000 mg/kg bw (92.8:7.2 <i>syn:anti</i> )                                                                |
| Rat, LC <sub>50</sub> , inhalation                 | > 5.28 mg/l (69.7:30.3 <i>syn:anti</i> )                                                                   |
| Rabbit, dermal irritation                          | Not irritating                                                                                             |
| Rabbit, ocular irritation                          | Slightly irritating                                                                                        |
| Mouse, skin sensitization (local lymph node assay) | Sensitizing potential                                                                                      |

### *Short-term studies of toxicity*

|                                  |                                              |
|----------------------------------|----------------------------------------------|
| Target/critical effect           | Body weight changes and liver toxicity (rat) |
| Lowest relevant oral NOAEL       | 20.3 mg/kg bw per day (rat)                  |
| Lowest relevant dermal NOAEL     | No data                                      |
| Lowest relevant inhalation NOAEC | No data                                      |

### *Genotoxicity*

Not genotoxic

### *Long-term studies of toxicity and carcinogenicity*

|                        |                                                                 |
|------------------------|-----------------------------------------------------------------|
| Target/critical effect | Clinical chemistry, body weight (rat)                           |
| Lowest relevant NOAEL  | 5.5 mg/kg bw per day (rat)                                      |
| Carcinogenicity        | Unlikely to pose a carcinogenic risk at dietary exposure levels |

### *Reproductive toxicity*

|                                      |                                                             |
|--------------------------------------|-------------------------------------------------------------|
| Reproduction target/critical effect  | No reproductive effects                                     |
| Lowest relevant reproductive NOAEL   | 239.1 mg/kg bw per day (rat), highest dose tested           |
| Developmental target/critical effect | Decreased fetal body weights (rat), microphthalmia (rabbit) |
| Lowest relevant developmental NOAEL  | 20 mg/kg bw per day (rat), 150 mg/kg bw per day (rabbit)    |

### *Neurotoxicity/delayed neurotoxicity*

No evidence in acute or subchronic neurotoxicity studies

### *Other toxicological studies*

|                        |                                                                                                                               |
|------------------------|-------------------------------------------------------------------------------------------------------------------------------|
| Studies on metabolites | In rat 4-week feeding studies, CSCD465008 was less toxic than the parent and CSCD459488 was of similar toxicity to the parent |
|------------------------|-------------------------------------------------------------------------------------------------------------------------------|

### *Medical data*

No reports submitted

### *Summary*

|      | Value           | Study                             | Safety factor |
|------|-----------------|-----------------------------------|---------------|
| ADI  | 0–0.06 mg/kg bw | Two-year study in rats            | 100           |
| ARfD | 0.3 mg/kg bw    | Acute neurotoxicity study in rats | 100           |

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